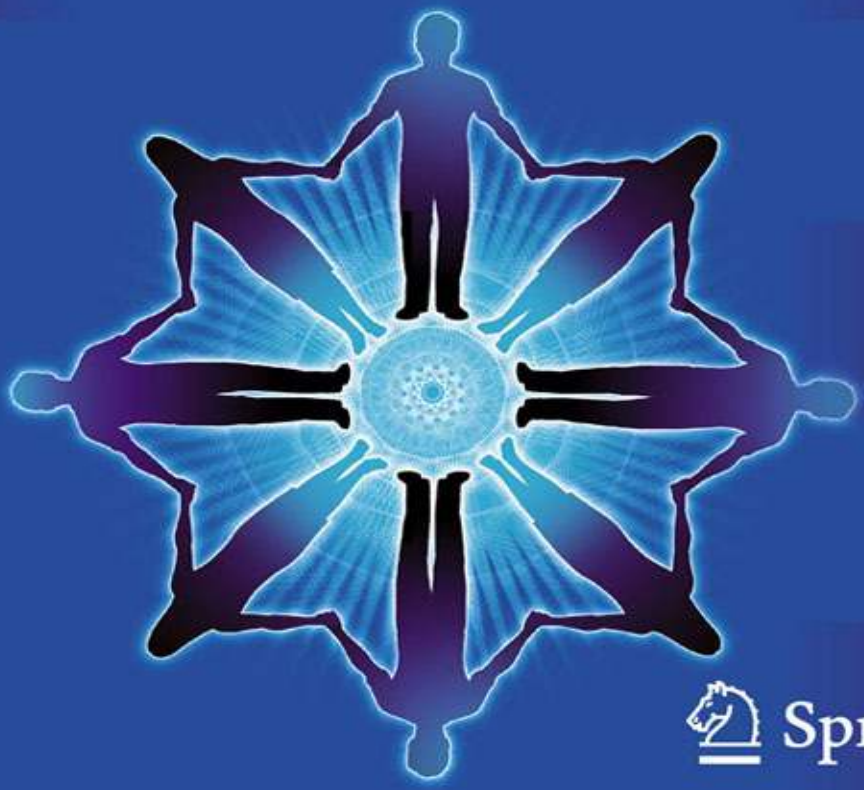
Philip S. Brachman • Elias Abrutyn
Editors

Bacterial Infections of Humans

Epidemiology and Control

Fourth Edition

Formerly edited by
Alfred S. Evans and Philip S. Brachman



Springer

VISIT…



Bacterial Infections of Humans

Epidemiology and Control

FOURTH EDITION

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Epidemiology and Control

FOURTH EDITION

Edited by

Philip S. Brachman
Emory University, Atlanta, Georgia, USA
and
Elias Abrutyn
Drexel University, Philadelphia, Pennsylvania, USA

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Biographical Sketch – Elias Abrutyn

Elias Abrutyn, co-editor of this, the 4th Edition of *Bacterial Infections of Humans*, died unexpectedly on February 22, 2007, at the age of 66 years. Eli was an extremely enthusiastic and energetic collaborator in developing this edition. His knowledge of infectious diseases of both literature and current activities in the field contributed significantly to the quality of this textbook.



Elias Abrutyn
1940–2007

Eli was born on April 15, 1940, in New Jersey and received his BA degree from the University of Pennsylvania in 1960. He attended the University of Basil in Basil, Switzerland from 1960 to 1963 and was awarded his MD from the University of Pittsburgh in 1966. He was an intern and medical resident at the Hospital of the University of Pennsylvania in Philadelphia from 1966 to 1968. He joined the Epidemic Intelligence Service at the Centers for Disease Control and Prevention in 1968 and worked in the Field Services Branch, Immunization Division, where he became the acting assistant director. In 1970, he assumed the Chief Medical Residency position at the University of Pennsylvania Veterans Administration Medical Center. In 1971, he was a post-doctoral Fellow in Infectious Diseases at the Hospital of the University of Pennsylvania. In 1972, he became an assistant professor of Medicine at the University of Pennsylvania School of Medicine and in 1984 was appointed an adjunct professor of Medicine at the University of Pennsylvania School of Medicine, a position he held until 1996. From 1982 to 1997, he was also a professor of medicine at the Medical College of Pennsylvania. From 1989 to 2005, he was a senior scholar in the Clinical Epidemiology Unit and then the Department of Epidemiology and Biostatistics at the University of Pennsylvania School of Medicine. From 1991 to 1996, he was associate dean for Veteran's Affairs at the Philadelphia Veterans Administration Medical Center. In 1996, he became professor of medicine with tenure at the Medical College of Pennsylvania Hahnemann University and then Vice Chairman of the Department of Medicine. From 1998 to 2000, he was also the interim chairman of the Department of Medicine at the Medical College of Pennsylvania Hahnemann University. In 2000, he became the Associate Provost and Associate Dean for Faculty Affairs at

Drexel University College of Medicine and the Allegheny University of Health Sciences. In 2002, he also assumed the position of Interim Chief of Infectious Diseases at Drexel University College of Medicine. Eli had his boards in Internal Medicine and Infectious Diseases.

He was a member of and held offices in a number of prestigious professional societies including the American College of Physicians, American Medical Association, American Federation for Medical Research, American Society for Microbiology, American Board of Internal Medicine, Association for Practitioners in Infection Control, Association of Professors in Medicine, Hospital Infections Society (England), Infectious Diseases Society of America, International Society for Cardiovascular Infectious Diseases, and the Society for Healthcare Epidemiology of America. Among his accolades, Eli was past president of the Society for Healthcare Epidemiology of America, a master of the American College of Physicians Society for Internal Medicine, a Fellow of the Infectious Disease Society, and a member of the council of the International Society of Cardiovascular Diseases. Additionally, he belonged to a number of local and regional societies. He was an associate editor of Annals of Internal Medicine and was on the editorial board of Infectious Disease Practice, and Clinical Performance in Quality Healthcare.

He was a consultant for a number of national and local organizations, including the chair of several National Institutes of Health committees, and on a committee to review a program for controlling emerging infectious diseases at the Centers for Disease Control and Prevention. He was a reviewer of manuscripts for 20 different scientific journals. He was a member of a number of national and local scientific committees and was the chairman of a large number of these committees.

Over the years, Eli had a number of grants, primarily dealing with infectious diseases. He was author and/or co-author of 103 publications in peer-reviewed journals, primarily reporting on infectious diseases, including urinary tract infections, infective endocarditis, and influenza. He also published on antibiotics, public health surveillance, hospital epidemiology, and health education. He wrote a number of editorials published in peer-reviewed journals. He was senior author of a book “Infection Control Reference Service” that is considered one of the authoritative texts in the field, in addition to being co-editor of this text book. He was author and co-author of 46 chapters in textbooks and newsletter articles dealing primarily with infectious diseases. He also wrote a number of commentaries and many abstracts, and gave presentations at national and international meetings.

Eli was well respected by his peers and enjoyed teaching on topics in internal medicine with emphasis on infectious diseases. He was a scholar, a careful researcher, well read on all infectious disease topics, and recognized as a leader. He will be missed by his students, colleagues, and friends.

Preface

In Memoriam of Alfred S. Evans

The third edition of *Bacterial Infections of Humans* is dedicated to Alfred Spring Evans, who died on January 21, 1996, 2 ½ years after a diagnosis of cancer. Al was the senior editor of this textbook, which he founded with Harry Feldman in 1982.

Al was a clinician, epidemiologist, educator, catalyst for biomedical research, historian, author, speaker, seeker of the truth, sincere friend of students, sports enthusiast, traveler, and truly a man of all seasons. He was a devoted husband to Brigette Klug Evans, father of three children, and grandfather of four.



Alfred S. Evans
1917–1996

Al was born in Buffalo, New York, on August 21, 1917, to Ellen Spring and John H. Evans, M.D., one of the United States's first anesthesiologists and an early researcher in the field of oxygen therapy. He received his undergraduate training at the University of Michigan; was awarded an M.D. degree in 1943 from the University of Buffalo; interned in Pittsburgh, Pennsylvania; and performed his medical residency at the Goldwater Hospital in New York City. He was in the United States Army from 1944 to 1946, assigned as a public health officer to a base in Okinawa, Japan. It was there that he met Drs. Albert Sabin and John R. Paul, who came to Okinawa to test a new Japanese encephalitis vaccine. Al was invited by Dr. Paul to come to Yale University to work on the identification of the cause of infectious mononucleosis. He accepted this invitation and became an associate professor of medicine in the Department of Medicine from 1946 to 1950. He was then called back to active duty in the Army and acted as chief of the Hepatitis Research Laboratory in Munich, Germany. It was there that he met his future wife.

In 1952, he joined the University of Wisconsin Medical School faculty and was professor and chairman of the Department of Preventive Medicine. In 1959 he also became director of the state Laboratory of Hygiene. He took a sabbatical leave in 1960 to study for his M.P.H. degree, which he received from the University of Michigan in 1961. In 1966 he accepted the position of John Rodman Paul Professor of Epidemiology at Yale University. Additionally, he became the director of the WHO's Serum Reference Bank at Yale. He remained active as a teacher and researcher until 1988 when he became an emeritus professor. He retired from Yale in 1994.

Al was dedicated to teaching both undergraduate and graduate students, and his expertise included a full range of topics in preventive medicine, public health, epidemiology (both applied and clinical), infectious diseases, and other related subjects. He was one of the founders and the first director of the Graduate Summer Session in Epidemiology, which was first held in the summer of 1965 at the University of Wisconsin. He remained on its faculty when it was held at the University of Minnesota from 1967 to 1987, and then at the University of Michigan School of Public Health from 1988 through the summer of 1994. During his 29 years of association with the Graduate Summer Session, he was not only a lecturer, primarily in epidemiology and infectious diseases, but was also an active participant on the Planning Committee and was the unofficial social chairman for the 3-week session.

Al was a popular lecturer who used expected and unexpected visual aids and injected humor throughout his lectures. He enjoyed teaching, and his students were eager recipients, greatly stimulated by his teaching, who frequently acknowledged his excellence with words, deeds, and appropriate awards, such as the Bedpan Award for Clinical Teaching given at the University of Wisconsin in 1952. As a teacher he constantly looked for new methods of presenting information to students. He was dedicated to the method of problem-oriented sessions and developed his own infectious disease case studies, which he would incorporate into his lectures. Additionally, he was a popular guest lecturer at universities and institutions in the United States and throughout the world. He was never too busy for his students and provided ongoing consultation concerning their training and careers.

His research ranged over broad fields of infectious disease as well as public health and epidemiology, with an emphasis on the Epstein–Barr virus and the relationships between cancer and infectious agents. He was the author or coauthor of more than 230 scientific, peer-viewed publications covering a broad area of topics, including such titles as "Sneezes, Wheezes, and Other Diseases" (1961), "Farm Injuries" (1958 and 1961), and "The Instant-Distant Infection" (1966). He was a medical historian, writing essays on John Evans, Austin Flint, and Max von Pettenkofer. He constantly promoted the incorporation of presentations on medical or public health history in the programs of various societies and training programs. He was an active supporter of Yale's historic Beaumont Medical Club.

Al firmly believed in the importance of accuracy in investigations, whether in the laboratory or in the field. His initial involvement in a field investigation incorporated comprehensive descriptive epidemiology that, when appropriate, was followed by methodical and carefully conceived analytic epidemiological methods. He was innovative in the use of observational field studies, which is reflected in his studies of the Epstein–Barr virus. Al constantly promoted the development and use of serum banks, as reflected by the WHO Serum Reference Bank at Yale. There are numerous examples of how this facility made significant contributions to Al's studies of infectious mononucleosis, other infectious diseases, and, more recently, HIV/AIDS. He made significant contributions to the concepts of disease causation and researched and reworked Koch's postulates. In this regard, he acknowledged the important contributions that Jakob Henle made to the topic and renamed Koch's postulates the Henle–Koch postulates.

He was author or coauthor of five well-received textbooks: *Viral Infections of Humans* (which in 1977 received The American Medical Writers' Association award for the best book written for physicians), *Bacterial Infections of Humans, Methods in Observational Epidemiology, Causation and Disease: A Chronological Journey*, and *Symposium on Latency and Masking in Viral and Rickettsial Infections*. He was also an editor of the *Yale Journal of Biology and Medicine*. He was a consultant to the WHO, the Pan American Health Organization, the United States Public Health Service, and the National Aeronautics and Space Agency and worked specifically with the space program in establishing the infection quarantine programs for the Apollo 13 space mission. He frequently consulted with numerous Ministries of Health in other countries, including Czechoslovakia, Kuwait, France, Spain, Taiwan, the Philippines, and Vietnam.

Al remained current with the medical literature, applied new technology in his own research, and brought appropriate new information into his lectures and writings. He constantly acquainted his colleagues and students with pertinent new written information culled from the current medical literature.

He belonged to and was an avid supporter of a number of societies, including the American Epidemiological Society, for which he served as secretary-treasurer (1968–1972) and president (1973); The American College of Epidemiology, of which he was one of the cofounders and later served as president

(1990); The Society of Epidemiologic Research; The Infectious Disease Society of America; The Society of Medical Consultants to the Armed Forces, for which he served as president (1983); and the American Public Health Association (where he was president of the Epidemiology Section). He was also a fellow of the American Association for the Advancement of Science.

Al received numerous prestigious awards and lectureships, including the Thomas Parron Lecturer and Award, University of Pittsburgh (1977); the Thomas Francis Lectureship, University of Michigan School of Public Health (1986); the Abraham Lilienfeld Lectureship, American College of Epidemiology (1986); the First Harry Feldman Memorial Lectureship, American Epidemiological Society (1987); the John R. Seal Award, The Society of Medical Consultants to the Armed Forces (1987); the W.B. Harrington Lecture and Award, University of Buffalo (1988); the Abraham Lilienfeld Award, Epidemiology Section, American Public Health Association (1990); and the Kass Lecturer, The Infectious Disease Society of America (1995). He was a renowned poet, but never published any of his works. He enjoyed reciting them in the classroom, in private discussions, and at social events.

During his life, he set standards to which others will aspire. The world is richer for the activities of Alfred Spring Evans, and we will all be better scientists and humanitarians because of his contributions.

The contents of this book were written by experts in the specific topics of each chapter, many of whom Al knew personally. The quality of the chapters reflects the multiple contributions that Alfred Spring Evans made to each of our professional lives.

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CHAPTER 1

Epidemiological Concepts

Alfred S. Evans[1]

1. Introduction

Epidemiology is the study of the distribution and determinants of health-related states, conditions, or events in specified populations and the application of the results of this study to the control of health problems.(1) It is a quantitative science concerned in infectious diseases with the circumstances under which disease processes occur, the factors that affect their incidence and the host response to the infectious agent, and the use of this knowledge for control and prevention.(2) It includes the pathogenesis of disease in both the community and the individual. For infectious diseases, one must study the circumstances under which both *infection* and *disease* occur, for these may be different. Infection is the consequence of an encounter of a potentially pathogenic microorganism with a susceptible human host through an appropriate portal of entry and usually involves a demonstrable host response to the agent. Exposure is the key factor, and the sources of infection lie mostly outside the individual human host, within the environment, or in other infected hosts. Disease represents one of the possible consequences of infection, and the factors important in its development are mostly intrinsic to the host, although the dosage and virulence of the infecting microbe play a role. These intrinsic factors include the age at the time of infection, the portal of entry, the presence or absence of immunity, the vigor of the primary defense system, the efficiency and nature of humoral and cell-mediated immune responses, the genetic makeup of the host, the state of nutrition, the presence of other diseases, and psychosocial influences. These factors that result in the occurrence of clinical illness among those infected have been called the "clinical illness-promotion factors,"(3) and many of them remain unknown. The host responses can include death, the classic clinical features of the disease, mild or atypical forms, subclinical and inapparent infections, and the carrier state, which may exist in the absence of a detectable host response. While the clinician is primarily concerned with disease, the epidemiologist is interested in both infection and disease. Infection without disease is a common phenomenon, so that a study limited to clinical illness alone would give an incomplete epidemiological picture and would be a poor basis for control and prevention.(4) A full understanding involves the pathogenesis of the process leading to clinical disease both in the community and in the individual.

The concepts of epidemiology in bacterial infections are very similar to those of viral infections as expounded in the companion volume, *Viral Infections of Humans*,(5) so there will be overlap and repetition in this volume. Some of the differences between viral and bacterial infection include the intracellular position of all viruses, their smaller size, the requirement of living tissues for viral multiplication, the ease with which many viruses are spread by respiratory routes or by insect vectors, the relatively high order of immunity following viral infection, the usefulness of serological tests for the diagnosis of most viral infections, and the failure of viral infections to respond to antibiotic therapy. Highly sensitive and specific molecular methods are being increasingly employed to define the agent and the host response to it.(6–8)

Many concepts and methods of epidemiology apply to both infectious and noninfectious diseases, and there should be no essential dichotomy between the two.(9) In general, epidemiology can be regarded as the development, pathogenesis, and expression of infection and disease in a community in much the same way as clinical

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It should be noted that this chapter is the original introductory chapter as written by Alfred S. Evans for the first edition in 1982.

[1]Deceased. Dr. D. Scott Schmid, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, assisted in revising Section 10, Immune Response, after Dr. Evans' death.

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medicine is concerned with the development, pathogenesis, and expression in the individual. This book will attempt to cover both these aspects. While the "epidemiology of infectious diseases" has disappeared from the curriculum of many schools of medicine and public health in developed countries, the current epidemic of the acquired immunodeficiency syndrome (AIDS) has reawakened interest in the subject. In addition, the emergence of new diseases such as Legionnaires' disease, Lyme disease, the toxic shock syndrome and the development of antibiotic-resistant pneumococci and tubercle bacilli, the appearance of erythrogenic streptococci ("flesh-eating" streptococci) and a new cholera strain termed 0139, foodborne outbreaks of *Escherichia coli* O157:H7, and a large waterborne outbreak of cryptosporidiosis are among the "emerging infections" that continue to pose challenges to epidemiologists[10] and for which the Centers for Disease Control and Prevention (CDC) are developing preventive strategies.[11] It is becoming apparent that in developed countries, the continuing, and in some instances the increasing, importance of infectious diseases is causing concern among public health authorities.

In developing countries, infectious diseases are still a major cause of morbidity and mortality, and efforts are in progress to develop training programs in epidemiology and in surveillance in such areas. The Field Epidemiology Training Program of the CDC is a fine example of this effort. Several recent texts address the problems of surveillance.[12–16]

2. Definitions and Methods

2.1. Definitions

A working understanding of the terms commonly used in epidemiology and infectious diseases may be helpful to the student, microbiologist, and clinician unfamiliar with them. They are derived from those in *A Dictionary of Epidemiology*,[1] *Viral Infections of Humans*,[5] and the American Public Health Association handbook entitled *Control of Communicable Diseases Manual*.[17]

Attack rate or case ratio: This ratio expresses incidence rates in population groups during specified time periods or under special circumstances such as in an epidemic. It is often expressed as a percent (cases per 100). The *secondary attack rate* is the proportion of persons who develop infection within an appropriate incubation period after exposure to a primary case divided by the number exposed. The groups so exposed are frequently family members or persons located in an institution.

Carrier: A carrier is a person, animal, or arthropod who harbors a specific infectious agent in the absence of clinical illness with or without a detectable immune response. The carrier state may reflect carriage of the organism in the incubation period before clinical symptoms appear, during an apparent or inapparent infection (healthy or asymptomatic carrier), or following recovery from illness; it may be of short or long duration (chronic carrier), and it may be intermittent or continuous. Carriers may spread the infectious agent to others.

Case-fatality rate: Number of deaths of a specific disease divided by the number of cases $\times$ 100.

Cell-mediated immunity: This term has been used previously to designate immune mechanisms largely dependent on lymphocyte activity and in contrast to "humoral immunity." As T lymphocytes are now recognized as playing an important role in both, the term *T-cell immunity* is being more widely used.

Chemoprophylaxis: Administration of a chemical or antibiotic to prevent infection or to prevent the development of disease in a person already infected.

Colonization: Multiplication of an organism on a body surface (e.g., skin, epithelium, mucus membrane) without evoking a tissue or immune response.

Communicable period: Time during which a person (or animal) is infectious for another person, animal, or arthropod.

Endemic: This term denotes the constant or usual presence of an infection or disease in a community. A high degree of endemicity is termed *hyperendemic*, and one with a particularly high level of infection beginning early in life and affecting most of the population is called *holoendemic*.

Epidemic: An epidemic or outbreak is said to exist when an unusual number of cases of a disease occur in a given time period and geographic area as compared with the previous experience with that disease in that area. For diseases already present in the community, it is necessary to know the number of existing cases (prevalence) as well as new cases (incidence) to determine whether an increase has occurred. The definition of increases or excess cases is arbitrary and will vary from disease to disease. See Section 3 for further discussion.

Host: A person, animal (including birds), or arthropod in which infectious agents subsist or infect under natural conditions. In this book the term will most often refer to the "human host" unless otherwise stated.

Immunity: The specific resistance to an infectious agent resulting from humoral and local antibodies and from cell-mediated responses constitutes immunity. Immunity may be acquired through natural infection, by active immunization, by transfer of immune factors via the placenta, or by passive immunization with antibodies from another person or animal. The immune state is relative and not absolute, is gov-

erned largely through genetic control, and may be altered by disease- or drug-induced immunosuppression.

Immunodeficiency: A state representing impairment of the immune system of the host that affects its ability to respond to a foreign antigen. This may result from an inherited defect, or an acquired one such as a result of the disease itself, or of immunosuppressive drugs or an infectious agent that depresses the immune system. The human immunodeficiency viruses (HIV-1 and HIV-2) are the major examples of the latter.

Incidence rate: The number of new events (specific infection or disease) occurring in a given time period in a given population as the numerator and the number of susceptible persons in that population exposed to the agent as the denominator. This is usually stated as cases (or infections) per 100, 1,000, or 100,000. This rate may be adjusted for an age- or sex-specific numerator and denominator or any other characteristic of interest. Laboratory procedures may be required for numerator data on new infections, as measured by isolation of the agent or by antibody rises, or by both. They may also be required to identify those actually at risk in the denominator, i.e., those lacking antibody; other means of refining the denominator would be by eliminating adults in calculating rates of childhood diseases or eliminating those with a valid history of having had the disease.

Incubation period: The incubation period is the interval between exposure and the appearance of the first detectable sign or symptom of the illness. Ill-defined exposure to a source of infection or exposure to persons without apparent illness may obscure the starting point of the incubation period, and vague, premonitory, or prodromal signs of illness may obscure its termination point. The best estimate is often derived from single exposures of short duration to a clinical case or established source of infection (e.g., air, food, water, arthropod vector) and the development of the first characteristic or classic features of the disease. Experimental infections in volunteers give well-defined incubation periods, but these may not always be the same as under natural conditions. See Section 7 for further discussion.

Index case: This is the index or primary case of an illness in a family, group, institution, or community that may serve as a source of infection to others.

Infection: Infection represents the deposition, colonization, and multiplication of a microorganism in a host and is usually accompanied by an immune response. Infection may occur with or without clinical illness.

Isolation: This is a term applied to the separation of infected persons in such places and/or under such conditions as to prevent contact or airborne transmission of the infectious agent to others during the period of communicability. Infection control practice in hospitals as recommended by the CDC has divided isolation into two tiers of isolation precautions. The first tier is known as *standard precautions* and the second tier *transmission-based precautions*. Hand washing remains a prime preventive measure in regard to nosocomial infections.

Morbidity rate: An incidence rate in which the numerator includes all persons clinically ill in a defined time and population and the denominator is the population involved or a subunit thereof, usually expressed as the number of cases per 100,000 persons at risk.

Mortality rate: The same as morbidity rate except the numerator consists of deaths. This may be the total number of deaths in a population group (crude mortality rate, usually expressed as deaths per 1,000) or deaths from a specific disease (disease-specific mortality, usually expressed as deaths per 100,000).

Nosocomial infections: This term refers to infections that develop after entry into a hospital or other health care institutions and that are not present or incubating at the time of admission or the residual of an infection acquired during a previous admission.

Pathogenicity: The ability of an infectious agent to produce disease in a susceptible host. Some nonpathogenic agents can become pathogenic in an immunocompromised host such as persons infected with HIV.

Prevalence rate: The ratio of the number of persons in a defined population who are affected with the disease at any one time as the numerator and the exposed population at that point as the denominator. If this is based on the frequency of cases at a moment in time, then the term *point prevalence* is used. If it reflects the proportion of persons affected over a longer period, then the term *period prevalence* is employed. Most infectious diseases are acute and short lived, so that prevalence rates are not commonly used. The use of prevalence rates is more relevant to more protracted illnesses such as subacute bacterial endocarditis, tuberculosis, and leprosy, or to reflect carrier states that may persist for months or years. Prevalence rates reflect incidence times duration of disease. In seroepidemiological usage, the term *prevalence* denotes the presence of antigen, antibody, or another component in the blood.

Quarantine: The restriction of persons or animals exposed to an infected source during the incubation period for that disease to observe if the disease develops in order that other persons will not be exposed to the infectious agent during that period.

Reservoir: A person, animal, soil, or other environment in which an infectious agent normally exists and multiplies and which can be a source of infection to other hosts.

Surveillance: As concerns public health, surveillance is the systematic collection of data pertaining to the occur-

rences of specific diseases or health-related conditions, the analysis and interpretation of these data, and the dissemination of consolidated and processed information to contributors to the program and other interested persons for purposes of control and/or prevention (see Chapter 2 for detailed discussion and Cutts et al.[12] for a World Health Organization definition, as used in the Expanded Programme in Immunization). *Serological surveillance* is the identification of current and past infection through measurement of antibody or of antigen in serum from representative samples of the population or other target groups.

Susceptibility: A state in which a person or animal is capable of being infected with a microorganism. The lack of specific protective antibody usually indicates susceptibility to that agent, although reactivation or reinfection to some agents may occur in the presence of antibody.

Transmission: The mechanism by which an infectious agent is spread to another host (see Section 5).

Virulence: A measure of the degree of pathogenicity of an infectious agent as reflected by the severity of the disease produced and its ability to invade the tissues of the host.

Zoonosis: An infection or infectious disease transmissible under natural conditions from animals to man. It may be *endemic* (enzootic) or *epidemic* (epizootic).

2.2. Methods

Epidemiology can be divided into descriptive, analytical, experimental, and serological epidemiology. The major analytic methods in use are the cohort (prospective) and case–control (retrospective). This section will briefly present these concepts. For more detailed descriptions, textbooks and recent articles of epidemiology are recommended.[18–21] A textbook on epidemiological methods that includes discussion and examples in infectious disease[18] is recommended before undertaking an epidemiological study. An excellent brief book on all aspects of epidemiological studies has been published by the World Health Organization (WHO).[22]

2.2.1. Types of Epidemiological Studies. Epidemiological studies may be descriptive or analytical. Descriptive studies are based on available data sources and describe the patterns of disease in population groups according to time, place, and person factors. Epidemic investigations begin with a descriptive study. These data often suggest clues to the etiology of the condition or to the risk factors involved. Analytical studies are then designed to test the hypotheses of causation developed from the descriptive studies and usually require new data to do so. Three common analytical methods are employed in pursuing epidemiological studies.

2.2.1.1. Cohort Study. This is the most definitive and expensive type of study and is based on identifying a group or groups of persons (cohorts) who are followed over time for the development of disease (or infection) in the presence or absence of suspected risk factors that are measured at the start of the study. These studies are usually carried out by identifying a cohort or usually two or more cohorts at the present time and then following them longitudinally over time. One cohort will be the group exposed to a risk factor or there may be several cohorts, each with a different degree of exposure. There usually is another cohort of unexposed persons who are followed in the same way. The cohorts are followed until the effect of the exposure occurs or the study is terminated for other reasons. If the occurrence of disease is the expected outcome, persons immune to the disease at the beginning of the study would not be included in a study cohort. This has been called a *prospective cohort study* or simply a *prospective study*. In infectious disease epidemiology, it may be possible to identify the persons in the cohort who are susceptible or immune at the start of the study by measuring the presence or absence of antibody in the initial serum specimens. Serial serum samples are then taken in which the appearance of antibody indicates the approximate time at which infection occurs. The occurrence of clinical disease at the same time provides information of the clinical–subclinical ratio. If the appropriate serum samples are taken and frozen, the actual testing can be delayed to the end of the study.

An alternative method of conducting a cohort study is to identify a group of persons at some time in the past who were presumably free of the disease under investigation at that time, as indicated by examining existing records. The cohort is then followed to the present, or even beyond, by measuring the occurrence of infection (by serological tests) or disease in that defined population. This approach is called a *historical cohort study* or a *retrospective cohort study*. Because the case–control study is also retrospective in terms of the time when the observations are made, it must be distinguished from the historical cohort study.

In a cohort study the statistical methods involve the calculation of the relative risk of the prevalence in the ill individuals as compared with the controls. This can be calculated using the format of a fourfold matrix as depicted in Table 1.

Table 1. Matrix for Calculating Relative Risk Ratios

	Number of persons		
Characteristic or factor	With disease	Without disease	Total
Present	a	b	$a + b$
Absent	c	d	$c + d$
Total	$A + c$	$b + d$	

If the frequency, $ala + b$, of the characteristic in persons with the disease (a) in this total group ($a + b$) is statistically significantly greater than the frequency of the characteristic in those without the disease ($c/c + d$), then an association may exist between the characteristic and the disease. For further details of the mathematical and epidemiological techniques and the biases involved in the selection of cases and controls, readers are referred to recent texts such as *Methods in Observational Epidemiology*.[18]

2.2.1.2. Case–Control Study. This has been called a retrospective study because it studies persons already ill with the disease and compares their characteristics with a control group without the disease for the presence or absence of certain possible risk factors. When a significant difference in the prevalence of a characteristic or risk factor is found, then the possibility of a causal association is suspected. Further studies using the cohort method are then often carried out to add strength to the association. The case–control study is usually the first type made because it is based on existing data, can be completed in a relatively short time, and is the least expensive. However, it cannot define the true incidence of the disease in relation to the various factors because the denominator at risk is not known.

A variation of the case–control study, and one encompassing the concept of a cohort study, is termed the *nested case–control study*. In this a large cohort is studied either prospectively or retrospectively for the occurrence of a specific disease, then these cases are matched by age and sex with persons in the original cohort who did not develop the disease. Various attributes of the two groups defined at the start of the study can then be compared. This method is very useful for diseases or conditions of low frequency in which analysis of the entire cohort would be an overwhelming task. An example of this is a recent study of Hodgkin's disease in relation to elevated levels of antibody to Epstein–Barr virus (EBV).[23] In this analysis, 240,000 persons whose sera had been collected and stored in four serum banks were followed for 5 years through cancer registries or hospital records for the development of Hodgkin's disease. Forty-three cases were identified in this manner and the EBV and other antibody levels were determined in sera from the group and compared with results from matched controls bled at the same time. A significant increase in certain EBV antibodies over those of controls was found 3–5 years preceding the diagnosis of Hodgkin's disease.

Biases may occur in case–control studies in the selection of cases, in the selection of controls, and in the elucidation of data by interview or records concerning the characteristics in question in both cases and controls. The selection of cases should ensure that they are representative of all patients with that disease. Ideally, this would assume that all patients with the disease seek medical attention, that the correct diagnosis is made and substantiated, that all medical facilities are canvassed, and that all cases are detected. In practice, these criteria are seldom met, and patients, for example, from a single hospital may be the only ones studied. This introduces a bias, since certain patients may be excluded from a given hospital because of such factors as age (e.g., no pediatric wards), socioeconomic level, or military or civilian status; the patient or physician may select a given hospital because of nearness, religious affiliation, the physician's privileges, nature of payment, or other considerations. These patients are not representative of all patients with the disease. The presence or absence of the characteristic under study may also influence the selection process for either the case or the control group or both, giving spurious associations.

Biases also are common in the selection of controls. Usually, controls should be selected from the same population group from which the patients are drawn and should be closely comparable to the cases in all known characteristics (age, sex, socioeconomic level, ethnic groups) except the one under study. Random selection from a large group may equalize those differences, but usually groups matched for certain variables or individuals matched carefully for paired comparisons are selected. However, if the two groups are matched too closely, the association between the cause and its effect may be masked. In a hospital setting, ill patients with diseases other than those under study are sometimes chosen. Bias may occur if some of these patients have diseases that are influenced by the characteristic in question. To limit this, patients with noninfectious diseases are often chosen in an infectious disease study. In a community setting, healthy controls may be advantageous. In matching, only those variables known to affect the disease should be selected. Each matching factor included, while controlling the results, eliminates the possibility of evaluating that factor itself.

To avoid bias regarding the presence or absence of a characteristic in the procurement of data by interview or from records, those charged with data collection should not know which is case or control, and the ascertainment should be uniform or standard. Once the data have been obtained, the odds ratio associated with a given characteristic is calculated from Table 1 by the cross-product of $a \times d$ divided by $b \times c$. This estimate is based on the assumption that the frequency of the disease in the population is relatively small and that cases and controls are representative of their respective ill and non-ill populations for that disease. Examples of case–control studies for an infectious disease would include the influence of some characteristic such as genetic makeup (HLA type), smoking, preexisting disease, or socioeconomic level as a

risk factor in a given disease. It should be emphasized that a particular risk factor might operate at different levels or at several levels: It might affect exposure and infection, the severity of illness after infection has occurred, the duration of disease, the development of complications, or the case-fatality rate.

The advantages of case–control studies as compared with cohort studies (see Table 2) include the relatively small numbers of subjects needed, their relatively high efficiency, the shorter time to complete the study, and their suitability for diseases of low incidence. Their disadvantages include difficulty in finding the needed information about the characteristic in question, or the inaccuracy of the information; bias in obtaining data; and bias in the selection of cases and controls. The appropriate selection of the control groups is probably the most difficult task.

2.2.1.3. Cross-Sectional or Prevalence Study. This third type of investigation examines the occurrence of disease and of suspected risk factors in population groups at a point in time or over a relatively short period of time. Prevalence rates among those with and without the exposure are determined and compared. This approach is usually limited to diseases of slow onset and long duration for which medical care is often not sought until the disease has progressed to a relatively advanced stage. Thus, the risk factors present at the start of the disease may be difficult to identify. This method is used for certain chronic diseases, such as osteoarthritis, chronic bronchitis, and some mental disorders,[24] but it may also be useful in certain infectious diseases such as those occurring in a hospital setting.

The reader should review more detailed descriptions of epidemiological methods such as found in Refs. 18–22 before undertaking any type of epidemiological study, as well as consult with a statistician in the planning stage to ensure the validity of the procedures and the adequacy of the number of subjects involved. See Section 5 for discussion of methods in epidemic investigation.

2.2.2. Experimental Epidemiology. In infectious diseases, this represents planned experiments designed to control the influence of extraneous factors, among those exposed or not exposed to an etiologic factor, preventive measure, or environmental manipulation by the investigator. One example is the planned introduction of an infectious agent in a controlled fashion into a group of animals or volunteers and the analysis of the spread of infection and disease within these groups as compared to a non-exposed group. Such studies offer the most scientifically controlled method of epidemiological study. Unfortunately, many bacterial species or agents may not induce infection or disease in animal models. Certain susceptible animals (marmosets, chimpanzees) may not be available for study or are too expensive. Volunteers are very difficult to utilize in today's

Table 2. Some Features of Cohort and Case–Control Studies

Features	Cohort	Case–control
Approach	Identify the subsequent incidence of disease in persons with or without given characteristic(s)	Identify the presence or absence of characteristic(s) in persons with or without a given disease
Starting point	Persons with or without certain characteristics	Ill persons and controls (healthy or with other disease)
Measurement	Incidence of infection or disease or both	Prevalence of characteristic
Type of observation	Serial, longitudinal surveillance of entire group for development of infection or disease or both	Single analysis by interview, records, or a laboratory test of the characteristic in persons with and without disease
Advantages and disadvantages		
Incidence	Can be measured directly	Not measurable directly
Risk	Direct assessment	Indirect assessment
Disease spectrum	Can be measured from infection to mild and severe disease in relation to characteristic(s) and to other diseases	Not measurable; a clinical case is the starting point
Factor(s) or characteristic(s)	Defined before disease develops	Factor(s) defined after disease develops
Bias	Little, usually, since information is recorded before the outcome is known, but problems in ascertainment, diagnosis, and follow-up may create bias	Bias may be present in interviewer, in patient, and in control; data from records may be incomplete
Attrition	Individuals may be lost to observation or refuse to be studied	Cases and controls may die prior to completion of study
Time	Often long period of observation	Can be short
Efficiency	Low except for diseases of high incidence	Comparatively high
Sample size	Large, depending on incidence of the infection or disease	Relatively small

ethical, legal, and social environment, and there are good reasons for these restrictions.

2.2.3. Serological Epidemiology. The systematic testing of blood samples from a defined sample of a target population for the presence of antibodies, antigens, genetic markers, specific cell-mediated immunity, and other biological characteristics is called a *serological* or *immunological survey*. It constitutes an important epidemiological tool. Serological techniques can (1) identify the past and current prevalence of an infectious agent in a community; (2) identify the incidence of infection by seroconversion or a rise in titer in samples obtained at two different times; (3) reveal the ratio of subclinical to clinical infections, when combined with clinical data; and (4) determine the need for immunization programs and evaluate their effectiveness as to the presence, level, and quality of antibody produced, its duration, and the degree of protection against disease. Serological techniques are useful in defining the incidence, clinical importance, and spectrum of illness of a new agent such as *Legionella pneumophila*. The presence of antibody or antitoxin to diphtheria, pertussis, and tetanus, as determined in a serological study, is a good reflection of the level of immunization and public health practice in a community. This is especially true of tetanus, since antitoxin is acquired almost solely through immunization and rarely, if at all, through natural infection. The use of serological surveys in areas where medical care, diagnostic facilities, and reporting practices are inadequate may provide information essential for the control and evaluation of immunization programs. The uses, advantages, and disadvantages of serological surveillance and seroepidemiology for viral infections are presented in Chapter 2 of the companion book[5] to this volume.

Seroepidemiology is more widely applicable to viral than to bacterial diseases because of the wider occurrence of demonstrable antibodies in viral than in bacterial infections and because of the better means to measure them. Nevertheless, these techniques have proved useful in variable degrees for brucellosis, cholera, diphtheria, legionellosis, leptospirosis, *Mycoplasma pneumoniae* infections, pertussis, Q fever, Rocky Mountain spotted fever, syphilis, tetanus, tularemia, and typhoid fever. Specific mention of their applicability will be found in the relevant chapters of this book. The development of monoclonal antibodies and diagnostic techniques, such as the enzyme-linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA), permits highly specific, sensitive, and rapid serological diagnoses. Development of sensitive and simple DNA probes and the ability to amplify DNA by the polymerase chain reaction (PCR) provide tools not only for diagnostic microbiology but also for the identification of antigens in stored paraffin or frozen sections.[6]

3. Epidemics and Their Investigation

Detailed descriptions of the concepts and methods of epidemic investigation can be found in a number of articles and book chapters.[24–26] An excellent recent article discussing the use of the case–control method in epidemic investigation gives a thorough review of the subject and an extensive table of examples.[27] This section will only deal with the highlights of epidemic investigation.

3.1. Pathogenesis of an Outbreak

Three essential requirements for an outbreak of an infectious disease are (1) the presence or introduction of an infectious agent by an infected human, animal, bird, or arthropod vector, or its occurrence in air, water, food, soil, or other environmental source, or its presence in or on a fomite; (2) an adequate number of susceptibles; and (3) an effective means of transmission between the two. Five circumstances in which epidemics occur can be mentioned: first, when a new group of susceptibles is introduced into a setting where a disease is endemic; second, when a new source of infection is introduced into an area from which the microbial agent has been absent and many susceptibles are therefore present, as in the return of visitors from a foreign country, or the arrival of new immigrants, or the contamination of food, water, or other sources of exposure by an agent not normally present; third, when effective contact is made between a preexisting infection of low endemicity with susceptible persons as a result of changes in social, behavioral, sexual, or cultural practices. Crowding as in a prison camp or institution or exposure of a new portal of entry are examples. A fourth possibility is an increased susceptibility to infection or disease or both through immunosuppression or other factors that influence the host response, such as a preceding viral infection, nutritional disorder, treatment with immunosuppressive drugs, or presence of a chronic disease. The devastating effect of HIV on the immune system has resulted in a worldwide epidemic of enormous and increasing proportions. A fifth circumstance might be the increase in the virulence or dosage of a microbial agent. This may have accounted for the massive outbreak of waterborne *Cryptosporidium* infection that occurred in Milwaukee, Wisconsin, in March–April 1993, which involved 404,000 infected persons of whom 4,400 required hospitalization.[28]

Epidemics or outbreaks are often classified from the standpoint of the source of infection. A common-source or common-vehicle outbreak may result from exposure of a group of persons to a single source of infection. This could be an exposure to a common source occurring at a single point in time, as in most foodborne outbreaks (point

epidemics), and characterized by a sharply defined and limited epidemic curve, often within the incubation period of the disease. It could be exposure on a continued or extended basis, as would be the case from a contaminated water supply or air source (airborne) and would result in an extended epidemic curve. In the latter setting, variations in the epidemic curve could result from differences in the dosage and time of occurrence of the microbial contaminant in the common vehicle or in the amount consumed, or they could result from changes in the frequency of exposure of the persons at risk to infection. The secondary spread of certain infectious agents from human to human in a common-source outbreak will also alter the epidemic curve, often producing a group of scattered cases after the initial epidemic wave subsides; these are called *secondary cases* and can lead to *tertiary cases*.

A second type of epidemic spread called *propagated* or *progressive* is due to multiplication and spread of an agent from one host to another. This is also called *contact spread* and may be direct, indirect, or by droplets (see Section 7). This is most often human-to-human spread, but could involve animal or arthropod intermediates. Here, the epidemic curve depends on the number of susceptibles, the degree of contact with an infected host, the incubation period of the disease, the mechanism of transmission, the portal of entry, and the infectiousness of the causative agent. In either common-source or propagated outbreaks, the epidemic decreases or stops when (1) the number of susceptibles effectively exposed to the source is diminished by natural attrition, by immunization, by antibiotic prophylactics, or by actual development of the disease itself; (2) the source of infection is eliminated; or (3) the means of transmission is interrupted.

3.2. Investigation of an Outbreak

The general strategy in the investigation of an outbreak includes establishing whether an epidemic actually exists, determining its extent, identifying the circumstances under which it occurred (e.g., time, place, person), evaluating its probable mode of spread, and initiating the steps to be taken for its control. Notification to appropriate health authorities should be made and help in epidemic investigation sought, if needed, from appropriate national or state disease control agencies; written reports should be prepared and distributed. News releases should be prepared to inform but not unnecessarily alarm the public. The epidemiologist, clinician, and laboratory experts all have roles in the analysis and management of an epidemic. The specific steps in epidemic investigations are presented in Table 3.

Table 3. Steps in the Investigation of an Epidemic

1. Determine that an epidemic or outbreak actually exists by comparing with previous data on the disease
2. Establish an etiologic diagnosis if possible; if not, define the condition epidemiologically and clinically. Collect materials for isolation and serological test, and data from sick and well-exposed persons
3. Investigate the extent of the outbreak by a quick survey of hospitals, physicians, and other sources and its basic epidemiological characteristics in terms of time, place, person, probable method of spread, and the spectrum of clinical illness. Prepare a spot map of cases and an epidemic curve. Call in outside help if needed
4. Formulate a working hypothesis of the source and manner of spread as a basis for further study
5. Test the hypothesis by determining infection and illness rates in persons exposed or not exposed to putative source(s) of infection by questionnaire, interview, and laboratory tests. Try to isolate the agent from the putative source(s)
6. Extend epidemiological and laboratory studies to other possible cases or to persons exposed but not ill
7. Analyze the data and consider possible interpretations
8. On the basis of the analysis, initiate both short- and long-term control measures
9. Report the outbreak to appropriate public health officials
10. Inform physicians, other health officials, and the public of the nature of the outbreak and the ways to control it

3.2.1. Determination of the Presence of an Epidemic. An epidemic or outbreak is usually defined as a substantial increase in the number of cases of a disease in a given period of time for that particular geographic area; e.g., an increase in the number of deaths from influenza and pneumonia that exceeds by 2 standard deviations the average experience for that week over the past 5 years is said to indicate an influenza outbreak.

A clinical diagnosis confirmed by laboratory findings is most important in determining whether a specific disease has exceeded the number of previously recorded cases. Unfortunately, a laboratory-proved diagnosis may be difficult or impossible to establish, especially early in an epidemic.

A clinical and epidemiological definition including the key features of the disease may be needed as a guide to reporting and for disease recognition until the etiologic agent is identified and appropriate methods are developed for isolation of the agent and/or its serological identification. Recent examples of the use of this type of definition are Legionnaires' disease, toxic shock syndrome, and AIDS. Even when the causative agent is known and laboratory tools for diagnosis are available, the disease may not be reportable, thus making comparison with past experiences impossible. On a practical level, any apparent concentration in time or geographic area of an acute illness of marked severity or with unique clinical features involving the respiratory,

gastrointestinal, skin, or central nervous system deserves evaluation. In the absence of a specific diagnosis, a simple working definition of a case should be established on the basis of available clinical and epidemiological data. It should be concise and clear-cut. The number of such cases should then be determined by a quick telephone or record survey of hospitals, clinics, and appropriate practicing physicians in the area. If the epidemic seems to be widespread, as with influenza, a random telephone survey of homes may give an estimate of the attack rate. The rate of absenteeism from key industries and schools may also help define the magnitude of the outbreak.

3.2.2. Determination of the Circumstances Under Which the Outbreak Occurred. This often involves two phases: a preliminary assessment based on available data and a more intensive investigation when the situation is better defined.

3.2.2.1. Preliminary Assessment. This is usually based on existing clinical records. In addition to the key clinical features, the age, sex, race, occupation, home and work addresses, unusual behavioral or cultural characteristics, date of onset, recent travel, and functions attended by others in the group in the recent past should be recorded. A time graph is drawn of the epidemic cases by date (and maybe time) of onset, from which the incubation period may be estimable. The time from the onset of the first cases to the peak may give a clue to this, as will unique or single exposures to the presumed source of infection. A spot map (place) may reveal clustering of cases or a relationship to a common source in the environment (food, water, air, anthropod). The data are analyzed to identify the persons at highest risk or some common denominator of risk and to postulate the most likely means of transmission. Early identification of a common-source outbreak is most important for instituting control measures. This might be a single (point) exposure, as in a food outbreak, or a continued exposure, as in a contaminated water supply. Person-to-person spread, airborne transmission, arthropod-borne spread, and zoonotic disease, especially of domestic animals, should be considered.

Appropriate materials for laboratory investigation should be collected early in the outbreak, such as throat washings, stool specimens or rectal swabs, blood for culture, and an acute-phase serum sample. A public health or hospital laboratory should be consulted in this endeavor. Since antibody to an infectious agent may already be present in many persons already ill in an outbreak, it may be desirable for baseline antibody levels to collect serum from other unexposed persons or from those incubating the disease, such as other family members or neighbors. A higher geometric mean antibody titer to a specific agent in ill compared to unexposed persons implicates that agent in the epidemic. Appropriate samples from the environment (water, food) or from possible vectors (mosquitoes, lice) should also be collected for isolating the agent.

On the basis of this preliminary assessment, a hypothesis of transmission may be formulated and recommendations for immediate control and isolation techniques made. Surveillance plans for identifying added cases may be drawn up, the appropriate environmental data assembled (water, milk, food, air), and questionnaires prepared for cases and controls (if a case–control study is to be conducted) in a fashion permitting easy analysis (marginal punch cards, computer). Standardized forms for foodborne outbreaks are available from state health departments and the CDC.

3.2.2.2. Intensive Study. This analysis should confirm or negate the hypothesis. The questionnaires prepared for this phase should include all possible circumstances under which the epidemic occurred and be administered to those ill, those exposed but not ill, and a comparable group neither exposed nor ill. Sera and other materials should be collected from these groups for antigen and antibody tests. The completed questionnaires may then be analyzed for comparison of attack rates (illness rate) in the three groups and as related to various risk factors. Antibody analysis of sera taken at the time of the outbreak and of those taken 2–3 weeks later may not only confirm the diagnosis but also identify the occurrence of infection in persons who were exposed but did not become ill. It may be possible to identify the specific nature of an ongoing outbreak by comparing the geometric mean antibody titer of those patients who are acutely ill with that of other patients already convalescing or by comparing the titer of those not exposed with that of those who are ill. More intensive study of the environment, insect vectors, and animal reservoirs may be needed. The analysis should include hypotheses to find the one that best fits the available data.

On the basis of this appraisal, control measures, including immunization programs, and other preventive measures should be initiated. Irrespective of whether the causative agent can be identified or not, the key element is the interruption of the chain of transmission. A written analysis of the epidemic should be given to the appropriate authorities. If no causative agent can be identified, then the acute and convalescent sera and material for antigen identification should be frozen for later study when new etiologic agents or laboratory techniques are discovered. A good example of the benefit of this procedure is its use in retroactively identifying several outbreaks of Legionnaires' disease that had occurred prior to the outbreak in 1976 in Philadelphia, from which the organism was first isolated.[29]

3.3. Example of Investigating a Foodborne Outbreak

An outbreak of illness characterized by diarrhea, abdominal cramps, and little or no fever involved 366 college students on February 24, 1966, in a new dormitory complex at the University of Wisconsin.[30] A quick assessment indicated that illness was confined to students who ate in three of six dining halls that served food from a common kitchen to 3,000 students. No other dormitories were involved, and the cases were sharply limited in time. The epidemic curve shown in Figure 1 indicates a peak incubation of 14 h. Stool specimens were obtained both from ill and healthy students and from food handlers. Samples of leftover items of food were not available; however, refrigerated samples of routinely collected food items were found for testing. Food menus for the preceding evening revealed that the three dining halls in which ill students had eaten offered a choice of fish or roast beef with gravy, whereas the other three dining halls had a choice of hamburger or fish; the other food items were common to all six dining halls. On the basis of this preliminary assessment, the hypothesis was formulated that this was a foodborne outbreak due to an agent with an average incubation period of about 14 h (range 10–20 h) that was present in or introduced into one or more of the food items served exclusively in the three dining halls where students became ill. The laboratory was notified of this as a guide to its tests. For more intensive investigation, questionnaires concerning foods eaten, time of onset, and the clinical symptoms were distributed to both sick and well students who ate in the three dining halls. They were returned by 366 ill and 740 well students, representing all the ill and two thirds of the well students. The clinical data indicated that the illness lasted less than 24 h and was characterized mainly by diarrhea; about half the ill students complained of abdominal cramps. Nausea, vomiting, and fever were rare. The average incubation period was too long for a staphylococcal toxin, and the clinical features would be unusual for *Salmonella* or *Shigella*.

An analysis of food items is given in Table 4. The evidence incriminating a food is usually based on the greatest difference in percentage ill between those who ate and those who did not eat a given food. In this outbreak, 69.9% of those who ate beef with gravy became ill compared to 4.9% who did not eat this item who became ill; furthermore, no one who ate beef without gravy became

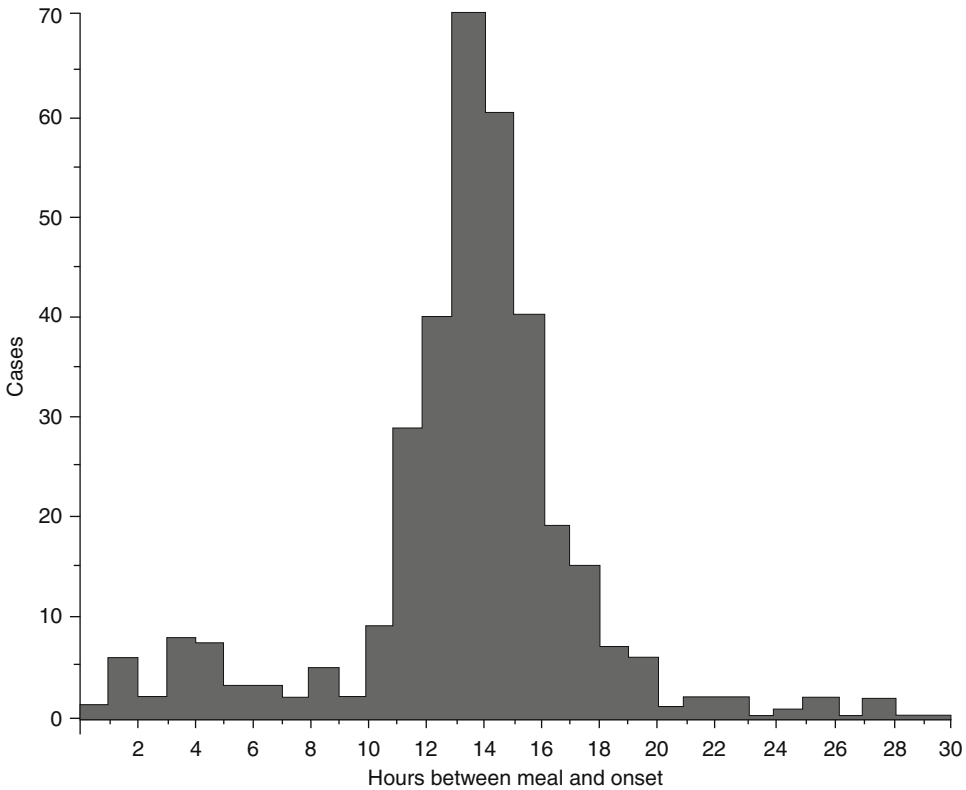


Figure 1. Epidemic curve of outbreak of *Clostridium perfringens* in a food outbreak involving 366 students at the University of Wisconsin. From Helstad et al.[30]

Table 4. Analysis of Attack Rate for Different Foods Eaten in a College Outbreak of Diarrhea[a]

	Ate food			Did not eat food			
		Ill			Ill		
Food item	Number	Number	Percent	Number	Number	Percent	Difference between ill and non-ill (percent)
Fish	391	16	4.1	715	340	47.6	–
Hamburger	188	15	8.0	918	351	38.2	–
Beef							
With gravy	479	335	69.9	627	31	4.9	65.0
Without gravy	48	0	0	1,058	366	34.6	–

[a]Derived from Helstad et al.[30]

ill, thus clearly incriminating the gravy as the likely source. The gravy as well as other foods available was negative on aerobic and anaerobic culture. Mouse-inoculation tests for toxin in the gravy were also negative; however, it was not known whether the gravy sample tested was from the incriminated meal or was set aside from a fresh gravy preparation. Laboratory analysis of fecal samples yielded the answer. Stool specimens from 19 of 20 ill students were positive for heat-resistant *Clostridium perfringens*, as was 1 of 24 stools from food handlers; no stools from 13 healthy students who had not eaten beef with gravy were positive. The organisms were isolated in thioglycollate broth after being heated 1 h in a boiling water bath. It was learned later that approximately 27 gal of bone beef stock had been kept overnight in the refrigerator in 9 gal plastic bags, mixed with 7 gal of fresh beef stock the next day, heated to a rolling boil, and served separately from the roast beef. Apparently, the inadequate heating of a heat-resistant preformed toxin in a very large volume of fluid had failed to destroy it. The control measures instituted were to prohibit future use of leftover gravy stock and to heat all items in smaller containers. *C. perfringens* food poisoning has an incubation period of 8–22 h with a peak of 10–14 h, closely fitting the outbreak described.

4. Agent

This book deals with microorganisms classified under "Lower protists (Prokaryotic): Bacteria." This grouping includes also the *Chlamydiae* (*Bedsoniae*) and the *Rickettsiae*, which are somewhat smaller than bacteria and are intracellular parasites.[31] Viruses, while classed as microorganisms, are sharply differentiated from all other cellular forms of life; they consist of a nucleic acid molecule, either DNA or RNA, that is enclosed in a protein coat or capsid. The principal groups of bacteria are presented in Table 5, which are derived from an "informal classification" presented by Jawetz et al.[31] in their excellent *Review of Medical Microbiology*, to which readers are to referred for discussions of microbiology, immunology, and host–parasite relationships. The new, beautifully illustrated book, *Medical Microbiology*, by Mims et al.[32] is also highly recommended.

The characteristics of microorganisms of epidemiological importance include those concerned with transmission through the environment, the development of infection, and the production of clinical disease. Table 6 summarizes some of these characteristics of bacteria, which include species pathogenic for humans.

4.1. Characteristics of Organisms That are Involved in Spread Through the Environment

For the spread of infection, a sufficient number of organisms must enter and survive transport through the environment to reach another susceptible host. Resistance to heat, UV light, drying, and chemical agents is important for survival of bacteria in nature. Some organisms such as *Vibrio cholerae* and *L. pneumophila* can survive for months in water, even in distilled water; others, such as *Bacillus anthracis*, attain survival through highly resistant spores. Organisms capable of actual multiplication within the environment in soil, plants, food products, milk, and elsewhere have an advantage for survival. The capacity to infect a nonhuman host such as animals or birds, or to be transferred through an insect vector such as the *Rickettsiae*, offers alternative pathways for the persistence and spread of microorganisms.

4.2. Characteristics of Organisms That are Involved in Production of Infection

Once bacteria have survived transport through the environment or intermediate host to reach a susceptible human host, several features of the bacteria are important in

Table 5. Key to Principle Groups of Bacteria Including Species Pathogenic for Humans[a]

Characteristics	Genera
I. Flexible, thin-walled cells with motility conferred by gliding mechanism (gliding bacteria)	
II. Same as I but with motility conferred by axial filament (spirochetes)	*Treponema, Borrelia, Leptospira*
III. Rigid, thick-walled cells, immotile or motility conferred by flagella	
Mycelial (actinomycetes)	*Mycobacterium, Actinomyces, Nocardia, Streptomyces*
Simple unicellular	
Obligate intracellular parasites	*Rickettsia, Coxiella, Chlamydia*
Free-living	
Gram-positive	
Cocci	*Streptococcus, Staphylococcus*
Nonsporulating rods	*Corynebacterium, Listeria, Erysipelothrix*
Sporulating rods	
Obligate aerobes	*Bacillus*
Obligate anaerobes	*Clostridium*
Gram-negative	
Cocci	*Neisseria*
Nonenteric rods	
Spiral forms	*Spirillum*
Straight rods	*Pasteurella, Brucella, Yersinia, Francisella, Haemophilus, Bordetella, Legionella*
Enteric rods	
Facultative anaerobes	*Escherichia* (and related coliforms), *Salmonella, Shigella, Klebsiella, Proteus, Vibrio*
Obligate aerobes	*Pseudomonas*
Obligate anaerobes	*Bacteroides*, Fusobacterium
IV. Lacking cell walls (mycoplasmas)	*Mycoplasma*

[a]Derived from Jawetz et al.[31]

the initiation and development of infection. One is the *infectiousness*, expressed as the ratio: number infected/number susceptible and exposed. A second is *pathogenicity*, a term used to denote the potential for an infectious organism to induce disease. It can be expressed quantitatively as follows: number with disease/number infected. The determinants of pathogenicity include mobile genetic elements such as plasmids, bacteriophages, and transposons.[33] The pathogenic process is complex and represents sequence of events in which many components are involved.[34] The adherence of organisms to host surfaces is a highly specific and essential step for subsequent events to develop. Epithelial surfaces provide appropriate sites for many bacteria. *M. pneumoniae* and *Haemophilus influenzae* find attachment sites in the respiratory epithelium, *Neisseria gonorrhea* in the urethral epithelium, and many enteric organisms (*V. cholerae, E. coli, Salmonella typhosa, Shigella flexneri*) in intestinal or colonic epithelium. Pathogenic organisms must possess characteristics that protect them against such host defenses as mucus and phagocytic cells. These protective features of the bacteria themselves include polysaccharide capsules (pneumococci, *Klebsiella, Haemophilus*), hyaluronic acid capsules and M proteins (β-hemolytic streptococci), and a surface polypeptide (*B. anthracis*). Toxins and certain extracellular enzymes may be important in the establishment of infection and in spread through tissues. The latter include collagenase (*C. perfringens*), coagulase (staphylococci), hyaluronidases (staphylococci, streptococci, clostridia, pneumococci), strepto-kinase or fibrinolysin (hemolytic streptococci), hemolysins and leukocydins (streptococci, staphylococci, clostridia, gram-negative rods), and proteases (*Neisseria*, streptococci) that can hydrolyze immunoglobulins, such as secretory IgA.[34] These surface properties and enzyme production contribute to the invasiveness of an organism.

4.3. Characteristics of Organisms That are Involved in Production of Disease

Disease is a rare consequence of infection. Usually, the presence of microorganisms on various surfaces of the body, their colonization on or in diverse epithelial cells, and their multiplication are unattended by signs of clinical disease.

Table 6. Bacterial Characteristics of Epidemiological Importance[a]

Epidemiological aspects	Bacterial characteristics
1. Features involved in *spread* through the environment	Number of organisms released by infected host
	Resistance to physical environment (e.g., heat, UV, moisture)
	Ability to multiply within environment
	Ability to infect intermediate host or insect vector
2. Features involved in initiation and development of *infection*	Host range of organisms
	Genetic makeup and antigenic diversity
	Infectivity of organism
	Pathogenicity of organism
	Number of organisms entering host and the portal of entry
	Enzymes involved in spread through tissues
3. Features involved in production of *clinical disease*	Most characteristics under (2)
	Virulence of organism
	Invasiveness of organism
	Production of endo- and exotoxins
	Immunopathologic potential

[a]Host attributes are considered in Table 7.

As with most viruses, infection without disease is a common outcome. However, since infection is a necessary basis for disease (excepting ingestion of preformed toxin), the attributes of organisms that are involved in infection are also important in clinical illness (see Section 4.2 and Table 6). The term *virulence* is used as a quantitative expression of the disease-producing potential of a pathogenic organism. Molecular studies of the determinants of virulence include how agents enter epithelial cells, a property that may be carried in different ways. In enteroenvasive *E. coli* it may be on a large plasmid, whereas for *Yersinia pseudotuberculosis* it is by a small DNA segment of the bacterial chromosome.[35] This property may be exchanged between bacteria, making noninvasive bacteria invasive.

Some organisms may lose apparent virulence if their structural identity is too close to the host's carbohydrates (for which the term *camouflage strategy* has been suggested). The factors that result in disease in an infected person are also determined by the host, as discussed in Section 6. Those that relate to the organism are invasiveness, the production of toxins, and the induction of an immune response that usually is beneficial but sometimes is detrimental to the host.

Invasiveness does not always correlate with disease and a widely disseminated organism does not always induce illness, but the wide distribution of a pathogen and its contact with many cells provide the potential amplification of any detrimental host response. That wide dissemination and a large number of organisms, even in the bloodstream, do not inevitably lead to toxemia is exemplified by the minimal host response evoked by large numbers of *Mycobacterium leprae* bacilli in the blood in lepromatous leprosy.

Organisms that produce endotoxins or exotoxins are likely to produce disease, since most are toxic to cells and evoke inflammatory responses. The exotoxins liberated by many gram-positive bacteria cause local cell and tissue injury; some damage phagocytic cells and thereby facilitate the spread of the organism. Examples include *Clostridium welchii, Clostridium botulinum, C. tetanus, Corynebacterium diphtheriae, S. dysenteriae, V. cholerae, B. anthracis, Bordetella pertussis, Streptococcus pyogenes*, and *Staphylococcus aureus*.[36] Some exotoxins are directly responsible for the characteristic clinical features of the disease, some are antiphagocytic, and some promote spread in tissues. They cause little or no fever in the host. Endotoxins are an integral part of the cell wall of gram-negative organisms. They are liberated in soluble form both during bacterial growth and presumably during death and disintegration of the organism. Endotoxins are strong immunologic adjuvants. They may produce fever in man and many other vertebrates; the pyrogenic action is mediated by a product synthesized by monocytes ("endogenous pyrogen") that acts on the thermoregulatory center in the hypothalamus. Other endogenous agents, such as prostaglandins and catecholamines, may also play a role. The lipopolysaccharide (LPS) is the most important component of endotoxin and is composed of a core polysaccharide common to many gram-negative bacteria, an O-specific polysaccharide conferring virulence and serological specificity, and a lipid A portion, mainly responsible for toxicity.[35] The effects of endotoxins appear to be mediated through leukotrienes, prostaglandins, and cachectin/TNF. Cachectin, which is identical to tumor necrosis factor,[37] is indistinguishable functionally from lymphotoxin, a product of activated T cells. This protein is produced by macrophages and by T cells in response to bacteria, viruses, and parasites and also occurs during cancers, resulting, in order of increasing dosage, in the following sequence of events: inflammation, cytotoxicity, cachexia, organ failure, irreversible shock, and death. There is a synergism between it and interleukin-1 in this phenomenon. Monoclonal antibody to cachectin/TNF can inhibit these responses and glucosteroids can also prevent endotoxin deaths. The gene for cachectin has been identified, and when put into hamster ovarian cells and then into nude mice results in cachexia. Despite these deleterious effects, cachectin/TNF in small doses appears to be beneficial in serving as a growth factor for macrophages

and as a tissue remodeler, a role for which it may have been designed in nature. It is not clear whether all these effects are mediated directly by cachectin/TNF or through mediators released by them. With large doses of endotoxins, there is also an effect that produces vascular collapse and death. Unlike exotoxins, endotoxins are heat-stable and are not fully convertible to protective toxoids. Endotoxins are normally released by many gram-negative bacteria in the intestines of healthy individuals, presumably absorbed in small amounts, and degraded by the Kuppfer cells. This occurs without pathological consequences on a continual basis and has a beneficial effect in stimulating the development of the immune system in the immature individual.[33]

Exotoxins are excreted by living cells, are quite unstable to heat, and consist of polypeptides of molecular weights from 10,000 to 900,000. They are highly antigenic and result in high titers of antitoxin that can neutralize the toxin. This antigenic property is useful in immunization with toxins rendered nontoxic by formalin, heat, and other methods. Different toxins produce disease via different mechanisms.[31] *C. diphtheriae* toxin results in inhibition of protein synthesis and necrosis of epithelium, heart muscle, kidney, and nerve tissues. The toxin of *C. tetani* reaches the central nervous system by retrograde axon transport where it increases reflex excitation in neurons of the spinal cord by blocking an inhibitor mediator. *C. botulinum* exerts its effect on the nervous system by blocking the release of acetylcholine at synapses and neuromuscular junction. Gut organisms, such as *Clostridium difficile*, produce a necrotizing toxin that leads to antibiotic-associated colitis, and that of *S. aureus* stimulates neural receptors from which impulses are transmitted to medullary centers controlling gut motility. *V. cholerae* toxin binds to ganglioside receptors on the villi of the small intestine, leading to a large increase in adenylate cyclase and AMP concentrations and the resulting massive hypersecretion of chloride and water and the impairment of absorption of sodium that characterizes the severe diarrhea and acidosis of cholera. Other toxins, such as that of hemolytic lysogenic streptococci, result in the punctate maculopapular rash of scarlet fever.

The production of clinical disease through immunopathologic mechanisms is more important for viral than for bacterial infections, but there are some examples of the latter. In streptococcal infections, antibodies may develop against an unknown component of the organism that is antigenically similar to heart muscle, leading to myocarditis. Immune complexes may also form, deposit in the kidney, and result in glomerulonephritis. In infections due to *M. pneumoniae*, the production of "heterophile" antibodies against human O erythrocytes (cold agglutinins) occasionally leads to acute hemolytic anemia. In primary infections by *M. tuberculosis*, the pathological picture is dominated by a vigorous and persistent cell-mediated immune response to the invading organism. The inflammatory, pathological, and immunologic processes of the host culminating in disease may be detrimental both to the host and to the microorganism. A successful parasite is one that leads to the least host response. For a more detailed discussion of microbial virulence factors, readers are referred to recent texts on infectious diseases and microbiology[32,38] and to recent articles.[34,39]

5. Environment

The external environment provides the setting in which the agent and host usually interact and is the usual means of transmission between the two. The effects of the environment on the organism itself are discussed in Section 4.1. The environment also contains the physical and biological mechanisms required for spread. The former includes air, water, and food and the latter animal, bird, and insect vectors. For some bacterial infections, the primary host is not man but some other living creature in the environment. This includes anthrax, brucellosis, leptospirosis, Lyme disease, Q fever, bubonic plague, Rocky Mountain spotted fever, salmonellosis, and tularemia. For infections involving insect transmission such as louse-borne typhus, Rocky Mountain spotted fever, and bubonic plague, the humidity, temperature, vegetation, and other factors in the environment may play a central role in limiting the occurrence of the infection to well-defined geographic areas favorable to the vector. Through climatic factors, the environment exerts an influence on exposure of the host to microorganisms. Warm weather and tropical climates result in recreational and occupational exposures to water, sewage, swimming pools, wild animals, and insects; they promote spread of skin infections in unclothed persons with abrasions on their skin. Certain organisms grow or survive better in warmer environments, especially enteric organisms. Intestinal infections flourish under such conditions. Inadequate refrigeration leads to foodborne outbreaks. Epidemics of Legionnaires' disease appear to depend on a chain of warm weather events. These include appropriate temperature and humidity for the organism to grow in soil or water, the contamination of a water-cooling tower or air conditioner, and airborne carriage or propulsion of the organism to susceptible humans, frequently in an enclosed environment (e.g., hotel, hospital, institution, club); July and August are good months for this.

The colder winter months, as well as school seasons in temperate climates of the northern hemisphere, bring individuals into close contact in a closed environment, facilitating spread of infections by the respiratory route. This includes bacterial infections of the central nervous system such as the meningococcus, *H. influenzae*, and pneumococcus organisms as well as true respiratory infections such as pertussis, bacterial and mycoplasmal pneumonias, tuberculosis, diphtheria, and streptococcal pharyngitis–tonsillitis. The peak period of different disease varies from one season to another. For example, pertussis and *M. pneumoniae* infections tend to occur most commonly in the fall and streptococcal infections in the spring. In addition, longer-term temporal trends in infection and disease occur within the same environment (as discussed in Chapter 2, Section 8.2). These changes reflect the complex interplay of agent, host, and environment. New strains of the organism, environmental alterations, varying behavioral patterns of the host, and the degree of immunization practice may change the pattern from one season to another.

The particular environmental setting—the macro- or microenvironment—also influences disease occurrence and its clinical patterns. In hospitals, certain types of skin, wound, and urinary infections are common, and their severity may be enhanced in persons who are already ill with another disease or are receiving immunosuppressive therapy. Antibiotic-resistant organisms are frequent in this setting. In prisons and in institutions for the mentally retarded or ill, low levels of personal hygiene and crowding contribute to the spread of respiratory, intestinal, and skin infections. Exposure in meatpacking and slaughterhouses or tanneries, or travel to developing countries, involves the risk of infection by various bacterial agents within that environment. With regard to children, the day-care center is posing an increasing hazard for them, as well as their parents for certain infectious diseases, especially respiratory and intestinal infections.[40] However, for young children infection is much more common than disease. For example, among 10,860 case contacts of an index case of *H. influenzae* type b infection in day-care centers, none of whom had received rifampin chemoprophylaxis, no clinical disease developed over an average 60-day observation period, although the carrier state was common.[41] Thus, rifampin prophylaxis of contacts may be unnecessary in these settings, although some other investigators disagree with this. For HIV infection, the environments of the brothel, the bar, and the bathhouse have provided ready transmission of this and other sexually transmitted infections, as has the "shooting gallery" of intravenous drug abusers. Travel to or residence in developing countries may result in exposure not only to specific tropical infections such as malaria, schistosomiasis, and cholera but also to many common respiratory and intestinal infections that the traveler might not encounter in a more hygienic setting. Even the homebody working in the garden or walking in the woods near home in endemic areas may be exposed to the bite of the deer tick or related species and become infected with *Borrelia burgdorferi*, the cause of Lyme disease.

6. Host

The occurrence of infection depends on exposure to a source of infection and on the susceptibility of the host. The development of disease in an infected person depends largely on factors intrinsic to the host, although some properties of the organism itself influence this. Some of the host factors are presented in Table 7.

They have been called the "clinical illness promotion factors."[3] Exposure to a pathogenic organism depends on characteristics of the human host that result in contact with sources of infection within the environment or that promote person-to-person spread. The behavioral pattern of the individual at different ages brings varying types of exposure in different seasons, cultures, and geographic areas. Personal habits such as intravenous drug usage or sexual promiscuity are also determinants of exposure. The family unit comprises an important setting for exposure and spread of infectious agents. The genetic background, nutritional habits, cultural and behavioral patterns, and level of hygiene within families create common patterns of exposure. The number and age of family members and the degree of crowding within the home also affect the transmissibility of infection. Hospitalization or institutionalization brings new exposures in closed environments. Heightened person-to-person contact and a mixture of susceptibles with infected or infectious individuals (carriers) underlie the increased risk of military recruits, children in day-care centers, and residents of institutions. Streptococcal, meningococcal, *M. pneumoniae, H. influenzae* type b, and enteric infections are common in such settings.

Occupational risks involve special types of exposures in certain occupations. The worker in the abattoir or slaughterhouse, meatpacking industry, or even at the butcher block is at risk to brucellosis, tularemia, and various parasitic infections. The sewage and sugarcane worker and the swine handler are exposed to leptospirosis, the tannery worker to anthrax, and the hunter to tularemia. The hospital worker is at increased risk to a variety of infectious agents, of which HIV is currently of greatest concern, although the risk is

Table 7. Host Factors that Influence Exposure, Infection, and Disease

Factors that influence exposure	
Behavioral factors related to age, drug usage, alcohol consumption	Military service
Familial exposure	Occupation
Hospitalization, especially intensive care	Recreation, sports, hobbies
Hygienic habits	Sexual activity: hetero- and homosexual, type and number of partners
Institutionalization: nurseries, day-care center, homes for the elderly and mentally retarded, prisons and other closed environments	Socioeconomic level
	Travel, especially to developing countries
Factors that influence infection, and occurrence and severity of disease	
Age at the time of infection	Entry portal of organism and presence of trauma at site of implantation
Alcoholism	
Anatomic defect	Genetic makeup, especially influences on the immune response
Antibiotic resistance	Immune state at time of infection
Antibiotic in tissues	Immunodeficiency: natural, drug-induced, or viral (HIV)
Coexisting diseases, especially chronic	Mechanism of disease production: inflammatory, immunopathologic, or toxic
Dosage: amount and virulence of organism to which person is exposed	
Double infection	Nutritional status
Duration of exposure to organism	Receptors for organism on cells needed for attachment or entry of organism

extremely small if universal hospital infection regulations are strictly followed. Hepatitis B infection is also of concern, and HBV vaccine should be given to all hospital staff exposed to blood or blood products.

Race does not usually influence infection if exposure is equal, but the response to infection may vary, such as the increased severity of tuberculosis in blacks. However, the separation of ethnic origin from other cultural, socioeconomic, behavioral, and genetic differences is often impossible. Recreational pursuits and hobbies influence exposures in both internal and external environments. The homemaker who prepares home preserves improperly may expose the ingestor to botulism; the home gardener or farmer is at risk to tetanus or Lyme disease, and the outdoorsman to various infections of wild animals. The influence of gender on occupational exposures has become of little importance, since women have entered almost all work areas including military life and many hazardous occupations. On the other hand, pregnancy is attended by special qualitative risks to infection, more commonly viral than bacterial, to which the male is not heir. Streptococcal B infections and syphilis are of special concern to the pregnant woman and her baby. Differences between the sexes in the portal of entry of microorganisms may result in different patterns of infection and disease, as is true in male homosexuals practicing passive rectal intercourse, in which the risk of infection with HIV is greatly increased. Genital lesions and discharges from sexually transmitted infections may also increase this risk in both sexes.

The socioeconomic level of the individual or the community affects the frequency, nature, and age at the time of infection. In developing countries and lower socioeconomic settings, infectious diseases, especially respiratory and enteric, constitute a leading cause of illness and death. The socioeconomic status influences infection and disease through a complex interaction of hygienic practices, environmental contamination, nutritional status, crowding, and exposure to animal and insect vectors.

Travel is an increasingly important risk factor because it may bring individuals into new settings, especially tropical or developing countries. Enteric infections are common hazards, especially toxogenic *E. coli, Campylobacter*, amebiasis, shigellosis, typhoid fever, salmonellosis, yersiniosis, and giardiasis. Enterotoxigenic *E. coli* (ETEC) is the most common cause and was found in 42% of diarrheal episodes in Latin America, 36% in Africa, and 25% in Asia. Other causes are various *Shigella* and *Salmonella* species. *Campylobacter jejuni, V. cholera, E. histoliticia, Giardia lamblia*, rotaviruses, and Norwalk-like viruses.[42] Mixed infections also occur. Bismuth subsalicylate had reduced the incidence of traveler's diarrhea by 65% in one study,[43] and its use is suggested for periods of up to 6 weeks.

Once infection has occurred, a number of factors influence whether clinical disease will develop and determine its severity (Table 7, part 2). Most of these factors are intrinsic to the host, although the dosage, virulence, and antibiotic resistance of the infecting organism play a role (see Section 4), as does the portal of entry. Entry sites that are close to vital organs or that permit easy access to invasion of the bloodstream may result in more severe and complicated infections. Among the host factors, age at the time of infection is an important determinant of the frequency of clinical illness

and its clinical features and severity. The presence of chronic diseases or other infections are risk factors. HIV infection greatly enhances the risk of opportunistic infections, of the reactivation of latent agents, and of malignancy.

Transmission of infection to the fetus in utero may result in fetal death or congenital abnormalities as with *Treponema pallidum*. Infections of the newborn such as those due to *Streptococcus B, C. botulinum*, and *Chlamydia trachomatis* may be severe and fatal. As is true in many viral infections, bacterial infections in childhood are often subclinical and less well localized than in the adult. The concept of "streptococcosis" illustrates this (see Chapter 34). In the newborn infant, respiratory involvement from group A infections is uncommon, although group B streptococci may cause sepsis and meningitis. In the age group 6 months to 3 years, group A infections have insidious development and mild symptoms. In the older infant and preschool child, a nonspecific streptococcal group A illness may be characterized by low-grade fever, irritability, and nasal discharge, sometimes accompanied by anorexia and vomiting. Clinical diagnosis is difficult. In the school-age child, upper respiratory infections due to group A predominate, and over half are manifested by the classic features of acute streptococcal pharyngotonsillitis: sore throat, often with tonsillar exudate, pharyngeal edema, dysphagia, enlargement of the anterior cervical nodes, and systemic symptoms (fever, chills, malaise). Another 20% may have milder and less localized illness, and 20% more may have either mild or no illness.

In general, the highest mortality from infection occurs very early in life, when immune defense mechanisms are immature, and in old age when they may be deteriorating. The clinical response to infection may also be more severe in conditions that alter or depress immune defenses. These include infection with HIV, organ transplantation, immunosuppressive drugs, and preexisting chronic disease, especially of the specific target organ of the infection; occurrence of a viral, parasitic, or other bacterial infection preceding or accompanying the current illness; and the prior use of alcohol or tobacco. The vigor and efficiency of the immune response may alter the host either favorably by control of the infection or unfavorably by certain immunopathologic processes. Genetic traits influence both susceptibility and disease. Their role in regulating the immune response is an important but often ill-defined one in relation to the occurrence and severity of the clinical disease. In tuberculosis, clinical illness among those infected has occurred more commonly in monozygotic twins and zygotic twins, even when other factors are controlled.[44] The nutritional level also affects host resistance. Malnutrition (especially severe protein deficiency) adversely affects phagocytosis and other primary defense mechanisms, the development of the thymus, and the efficacy of cell-mediated immunity against infections such as tuberculosis. In general, antibody formation is not impaired. The precise role of nutrition and vitamins in infection and disease is not well understood. Immunity is also influenced by vaccination, whether active or passive.

In summary, host factors may be divided into three major stages: (1) those that lead to exposure, (2) those that lead to infection among those effectively exposed, and (3) those that lead to clinical disease among those infected. The concepts of a clinical illness-promotion factor that leads to clinical illness[3] and of other, protective factors that result in subclinical or inapparent illness have been discussed.[4] Many of them remain unknown and they remain an important challenge to epidemiologists, microbiologists, immunologists, and geneticists.

7. Routes of Transmission

The major routes of transmission of bacterial infections are listed in Table 8 in general order of their importance. Many organisms have several routes. The sequence of spread usually involves the exit of the organism from the infected host; transport through the environment via air, water, food, insect, or animal, with or without bacterial multiplication; and the entry of a sufficient number of viable organisms into an appropriate portal of a susceptible host to initiate infection. For most infectious agents, specific receptors on the cell surface are needed to permit attachment and multiplication of the organism. Table 8 is loosely divided into human, animal–insect, and inanimate sources of infection in order to follow infection from its source to a human host, but the arrangement is sometimes artificial for those infections that exist primarily in other species or for those organisms that can multiply or survive in the natural environment.

7.1. Respiratory or Airborne

Organisms infecting the respiratory tract are either airborne via droplet nuclei or transmitted via droplets that are not considered true airborne transmission (see Section 7.2). The sources of the organisms carried by the air (droplet nuclei) include animate sources, the respiratory tract or oropharynx of infected persons, or from infected lesions of the skin, or from inanimate sources such as from water-cooling towers, as with *Legionella* organisms. Their success in reaching a susceptible host depends on the number of organisms present, the particle size, the force with which

Table 8. Transmission of Bacterial Infection[a]

Route of exit	Route of transmission	Examples	Factors	Route of entry
1. Human source				
1.1. Respiratory	Respiratory droplets or droplet nuclei	Bacterial pneumonias	Close contact or airborne	Respiratory
	RS and fomites	Diphtheria	Carriers	Respiratory, skin?
	Nasal discharges	Leprosy	Household contact	Skin, repiratory
	RS → droplets	Meningococcus	Crowding, military recruits, carriers	Respiratory
	RS → air, fomites	Pertussis	Direct contact	Respiratory
	RS → air	Plague (pneumonic)	Pneumonic case	Respiratory
	RS → droplets	Streptococcal	Close contact, carrier	Respiratory
	RS → droplet nuclei	Tuberculosis	Household contact	Respiratory
1.2. Skin squames	Respiratory, direct contact	Nosocomial bacterial infections	Hospitalization, surgery	Nose, respiratory, skin
	Direct contact	Impetigo due to staph and/or strep	Low socioeconomic level, tropics	Skin
	Close contact	Skin diphtheria	War wounds	Skin
		Yaws	Endemic foci	Skin
1.3. Gastrointestinal				
Enteric fevers	Stool → water	Cholera	Water, food, carrier	Mouth
	Stool → food	Salmonellosis	Food, animal contact	Mouth
	Stool → man	Shigellosis	Man to man only	Mouth
	Stool → water, food	Typhoid fever	Also food, flies	Mouth
Food poisoning	Food	Staph, *C. perfringens, Salmonella*, strep, *Vibrio parahemolyticus*	Inadequate refrigeration or cooking	
		Botulism	Home canning	Mouth
1.4. Urine	Water (swimming)	Leptospirosis	Infected animals	Skin
	Water	Typhoid fever	Poor sanitation	Oral
1.5. Genital	Sexual contact (hetero- or homosexual)	Chancroid	Mostly tropics	Urethra
		Chlamydia	Also carriers	Urethra, rectum
		Gonorrhea	Also carriers	Urethra, rectum
		Syphilis	Moist surfaces	Urethra, placenta
1.6. Placental	Congenital	Syphilis	Up to fourth month of pregnancy	Blood
1.7. Umbilical	Direct contact	Neonatal tetanus	Poor birth hygiene	Cord
2. Animal sources	Infected animal	Anthrax	Tanning	Skin, respiratory
		Tularemia	Skinning, dressing	Skin, eyes
	Bite of tick	Rocky Mountain spotted fever, Lyme disease	Outdoor exposure	Skin
	Rat flea	Bubonic plague	Infected rat	Skin
	Infected placenta via air	Q fever	Cows	Respiratory
3. Inanimate sources	Soil, air, water, food	Tetanus	Wound, childbirth	Skin
		Legionnaires' disease	Warmth, humidity, water coolers, air conditioners, potable water supplies	Respiratory

[a]This table is representative only and does not include all organisms. RS, respiratory secretions.

they are propelled into the environment, the resistance to drying, the temperature and humidity of the air, the presence of air currents, and the distance to the host. Some infections may be carried great distances from their sources, e.g., Q fever, tuberculosis, or Legionnaires' disease. As with viruses, respiratory-transmitted bacterial infections are difficult to control. The size of the particles in the aerosol influences its dispersion distance and the site in the respiratory passages at which the particles are trapped. Particles larger than approximately 5 μm in diameter are usually filtered out in the nose, while those up to 5 μm in diameter are deposited on sites along the upper and lower respiratory tract.

7.2. Contact

Contact transmission can be direct, indirect, or by droplets, which due to particle size are not truly airborne. Direct contact transmission means the agent is transmitted from an infected person or animal directly to a susceptible host; indirect contact transmission means the agent moves from the source to the susceptible host by means of an inanimate object. Droplets are infected particles more than 5 μm in diameter that only travel up to approximately 3 ft through the air before they infect a susceptible host or fall to a horizontal surface. Direct contact spread includes genital or sexually transmitted diseases, some gastrointestinal or fecal–oral spread diseases, and some other diseases such as tularemia resulting from contact with an infected animal.

Bacterial infections of the skin are transmitted usually person to person from an infected lesion or via squamae. They are commonly due to staphylococcus or streptococcus or a mixture of the two. They are manifested as boils, carbuncles, impetigo, and erysipelas. They are particularly common in warm and tropical climates and in settings of poor hygiene. Yaws, a nonvenereal, contagious disease of the skin and bones due to *Treponema pertenue*, is endemic in many tropical areas and is similarly transmitted; effective eradication programs with penicillin sponsored by the WHO have been carried out in many countries. Diphtheritic skin infections may also occur, especially in tropical climates, and may contaminate wounds.

7.3. Genital or Sexually Transmitted

The term *venereally transmitted* is now being limited to the five classic infections clearly transmitted by sexual intercourse (gonorrhea, syphilis, chancroid, lymphogranuloma venereum, and granuloma inguinale). The newer term, *sexually transmitted diseases* (STD), is broader, applies to both hetero- and homosexual activities, and encompasses all infections transmitted person to person during sexual activity. In recent years, *C. trachomatis* has been identified in this group as the cause of almost half of nongonorrheal urethritis (see Section 11.2.6 and Chapter 9), and *Ureaplasma* is under evaluation; enteric infections are of increasing importance in male homosexuals. The presence of ulcers due to STDs enhances the spread of HIV, especially a penile lesion from syphilis or herpes simplex.

7.4. Gastrointestinal or Fecal–Oral

The fecal–oral route of transmission is a close rival in frequency to respiratory spread and there are many sources of infection. A first group is called enteric fevers. Bacterial organisms from ill persons or carriers exit via the gastrointestinal tract to the external milieu for transmission via water, food, or direct contact to another individual. They constitute a major group of bacterial infections. The mouth is the common portal of entry. Some enteric infections involve only a human-to-human cycle, such as cholera, typhoid fever, and shigellosis. Others, such as salmonellosis, *Campylobacter* infections, and yersiniosis, also involve animal hosts. A variety of mechanisms may transmit the organism from the infectious stool to a susceptible person. Cholera is commonly transmitted by water, and on occasion by food (such as undercooked seafood like oysters and clams). Shigellosis is spread by the fecal route from patients or carriers and also by food, water, or flies. Typhoid fever is transmitted by food or water contaminated by the feces or urine from a patient or carrier and sometimes through contaminated shellfish or canned goods. *Salmonella* organisms are widely disseminated in nature and infect many domestic animals and birds, providing many potential sources of contamination of food and, less commonly, of water. *Campylobacter fetus* subsp. *jejuni* and *Yersinia enterocolitica* also infect many animals, including domestic ones such as puppies, and can infect exposed humans through direct contact or through water, milk, or food. For shigellosis, exposure to an infected human during the acute illness or shortly thereafter is the main source of infection; here, direct or indirect fecal–oral contact is usually more important than water or food. There is no extrahuman reservoir of infection.

A second group of gastrointestinal infections is called food poisoning. Here, contamination of food may occur from the feces of an infected person or carrier (food handler), but other sources of the organism are also common. The animal food source may be infected (i.e., *Salmonella* in chickens), or the organism may be present on the skin of the food handler (staphylococcus, streptococcus), in the environment (staphylococcus), in the soil (*C. perfringens, C. botulinum*), or in raw seafood (*Vibrio parahemolyticus*).

The transmission of enteric fevers and food poisoning is largely preventable. A good source of water, proper chlorination or boiling, frequent hand washing, appropriate refrigeration of foods, and thorough cooking are effective ways of interrupting the chain of infection. However, in developing countries and low socioeconomic settings, neither the means nor the education to carry them out may be available. Some may be prevented by immunization. Transmission of enteric infections by homosexual activity is a newly recognized and important problem in this group, and infection in HIV-infected patients may result not only from a wide variety of usual pathogens such as *E. coli* but also from organisms usually regarded as commensal and nonpathogenic such as the parasitic infection *Cryptosporidium*. However, this

organism caused a massive outbreak related to the public water supply of the city of Milwaukee in normal hosts.[28] This organism and other related ones are able to pass through usual water filters and are highly resistant to the usual levels of control. Increased surveillance of their occurrence in wells and public water systems is currently being carried out in several states under the auspices of the CDC.

7.5. Urinary

Urinary spread of infection is not common, but may occur in typhoid fever from an infected person and in leptospirosis from many animal hosts. Water is the common vehicle of transmission.

7.6. Perinatal

These infections occur at the time of childbirth. In *congenital infections*, the organism is transmitted *vertically* from an infected mother via the placenta to the fetus. Congenital syphilis, rubella, and toxoplasmosis are examples of this. Infections may occur *horizontally* from an infected cervix to the baby as it passes through the birth canal, as in gonococcal ophthalmia and chlamydial infections. Infections may also be acquired immediately after birth, as exemplified by tetanus neonatorum due to contamination of the newly cut umbilical cord by soil or a contaminated substance applied to the umbilical stump.

7.7. Insect Vectors

Rocky Mountain spotted fever is transmitted by the bite of the tick, which may remain infective for a long time, and the infection is maintained in nature by transovarian and transstadial passage. A mite has been implicated as the means of transmission of rickettsialpox from infected house mice. The transmission of bubonic (sylvatic) plague is through the rat flea (mostly *Xenopsylla cheopis*) from infected wild rodents. *B. burgdorferi*, the cause of Lyme disease, is transmitted by small Ixodes ticks, such as the deer tick, *Ixodes dammini*.

8. Pathogenesis

A section on pathogenesis is included in every chapter of this volume that deals with specific infections. Only a few concepts will be presented here. An excellent book by Mims,[35] entitled *The Pathogenesis of Infectious Diseases*, should be consulted for details, as well as more recent articles by him[36] and others.[33,34] Recent texts of medical microbiology[32] and infectious diseases[38] also contain excellent discussions.

8.1. Localized or Superficial Infections

Many bacterial infections produce diseases through the cells with which they first come in contact in skin or epithelial surfaces and remain limited to that area. Tissue damage results from the direct action of the bacteria, microbial toxins, indirect injury, inflammation, or immunopathologic processes. Most bacteria have specific attachment sites on epithelial surfaces (see Section 4.2). Examples of localized infections include diphtheria and streptococcal infections of the throat, gonococcal infections of the conjunctiva or urethra, cholera, and most *Salmonella* infections of the intestine. Many gram-negative bacteria have a limited capacity to invade tissues and tend to remain localized; some are able to invade only in debilitated, malnourished, or immunocompromised patients. Host antibacterial forces limit the spread of many bacteria. At the subepithelial level, three important defense mechanisms are called into play: (1) tissue fluids, (2) the lymphatic system leading to the lymph nodes, and (3) phagocytic cells (macrophages) in tissues and polymorphonuclear cells in the blood. Each of these mechanisms depends on the inflammatory response for its action,[35] as manifested by four cardinal signs: *warmth* and *redness* due to vasodilatation, *swelling* (vasodilatation and exudate), and *pain* (tissue distention, pain mediators). Polymorphonuclear cells enter, as well as macrophages and lymphocytes; exudation occurs. Tissue fluids provide plasma proteins, including immunoglobulins, such as IgG, complement, and properdin. The primary mediators of inflammation include histamine, 5-hydroxytryptamine, and kinins. Prostaglandins E and F are thought to play a role in the termination of the response. Microorganisms in peripheral lymphatics are rapidly carried to lymph nodes, where they are exposed to macrophages lining the sinus that act as a bacterial filter. Here, too, polymorphs, serum factors accumulating during inflammation, and the initiation of the immune response limit the infection. The phagocytic cells play a key role in the interaction with microorganisms, ingesting and killing bacterial invaders. Among the chemotractant factors for phagocytosis are platelet-activating factors, leukotriene B4, C5a, and certain formyl peptides.[33] The details are fully described by Mims,[32,36] as are the ways in which some bacteria are able to resist or interfere with phagocytic activity. Organisms that escape must still face one or two encounters with the macrophages, as well as other immune mechanisms, before successfully reaching the venous system.

8.2. Systemic Infections

Organisms that escape phagocytic cells and the other local defense mechanisms can spread through the tissues and, more distantly, via the lymphatics and the bloodstream. Some viruses (herpes, HIV, poxviruses, measles), some rickettsiae (*Rickettsia Rickettsii, R. prowazekii*), and some bacteria (*M. tuberculosis, M. leprae, Listeria monocytogenes, Brucella* spp., and *L. pneumophila*) actually multiply in macrophages. The toxins, enzymes, and surface components of bacteria that protect them against phagocytic destruction and promote invasiveness have been mentioned in Section 4. It is not clear what exact role the proteinases, collagenases, lipases, and nucleases produced by bacteria play in the pathogenesis of infection or which ones are related to nutritional and bacterial metabolism.

Lymphatic spread may occur from the lymph node, which serves not only as a focus of phagocytic and immune forces but also, if these fail, as a focus of dissemination. These results occur when the lymph flow rate is high from inflammation of tissues or from exercise of muscles, when the number of bacterial particles exceeds the filtration rate or the defense mechanisms of the node or both, and when phagocytic activity is impaired. In some instances, certain organisms such as *Pasteurella pestis* and brucellosis actually multiply in the lymph node and spread via efferent lymph channels. In other instances, vigorous inflammatory responses localize the infection, and the node becomes a graveyard of dead and damaged bacteria and of tissue cells.

Bloodstream or *hematogenous spread* is the most effective mechanism for the dissemination of an infection throughout the body. Bacteria may exist free in the plasma (pneumococci, *B. anthracis, Leptospira*), intracellularly in monocytes (*Listeria*, tubercle and leprosy bacilli, *Brucella*), or in association with polymorphonuclear cells (many pyogenic bacteria). The *bacteremia* may be transient and with little or no systemic response, as follows dental extraction in a healthy person; even a continuous bacteremia may exist with few toxic signs, as in leprosy where the organism exists in large numbers inside blood monocytes. On the other hand, severe systemic manifestations may accompany the presence of large numbers of organisms in the blood such as the pneumococcus, meningococcus, or group A *S. pyogenes*. This is called a *septicemia*. Bacteria may succeed in setting up foci of infection in areas where the blood flow is slow enough, or they may establish multiplication in sites previously damaged by disease or injury, such as *Streptococcus viridans* on abnormal heart valves producing subacute bacterial endocarditis, or staphylococci in the traumatized long bones of children may lead to osteomyelitis. Depending on the site of the infection, the liver and lung may receive many organisms during bacterial invasion of the bloodstream. The lung, liver, spleen, and bone marrow may also serve as important foci of dissemination of organisms, as in brucellosis, leptospirosis, and typhoid fever. Rashes accompany the dissemination of many viral and some bacterial infections to the skin. They may result from localization and growth of the organism in small blood vessels, producing thrombosis, infarction, and hemorrhage as in the rickettsial diseases, Rocky Mountain spotted fever, and typhus, as well as the petechial and purpuric lesions of meningococcemia. Immunopathologic processes involving sensitized lymphocytes, antibodies, and immune complexes play a role in many rashes, especially viral. A bacterial toxin may induce the rash as in scarlet fever. Some organisms such as *T. pallidum* in secondary syphilis extravasate from blood vessels and multiply in extravascular tissues. This results in highly infectious lesions that discharge to the exterior. Dissemination of *T. pallidum* to the blood–fetal junction in the placenta during pregnancy may result in infection of the fetus; slow blood flow in the placenta may contribute to this possibility.

Central nervous system (CNS) and meningeal involvement can occur by bloodstream carriage of the organism to the blood–cerebrospinal fluid junctions in the meninges or choroid plexus; from there, passive transport occurs into the flow of fluid from ventricles to subarachnoid spaces and throughout the CNS. Examples of bacteria that traverse this barrier and produce meningitis are the meningococcus, tubercle bacillus, *L. monocytogenes*, and *H. influenzae*. Actual spread along peripheral nerves has been shown for rabies and herpesviruses and is the means of centripetal passage of tetanus toxin.[45]

9. Incubation Period

The period of time from exposure to a source of infection to the first signs or symptoms of clinical illness is called the incubation period (IP). It varies with (1) the nature and dosage of the organism; (2) the portal of entry; (3) the type of the infection (localized or systemic); (4) the mechanism responsible for tissue injury (invasion, toxin, immunopathologic process); (5) the immune status of the host, being prolonged in the presence of partial immunity; and (6) other unknown factors individual to the host. The IP has many uses in epidemiology: (1) it helps define the etiologic agent in an epidemic; (2) it helps differentiate common source from propagated epidemics and identify the reservoir and/or source of the agent; (3) it delineates the period for which a person exposed to an infection is at risk to development of disease; (4) it assists in identifying the period of

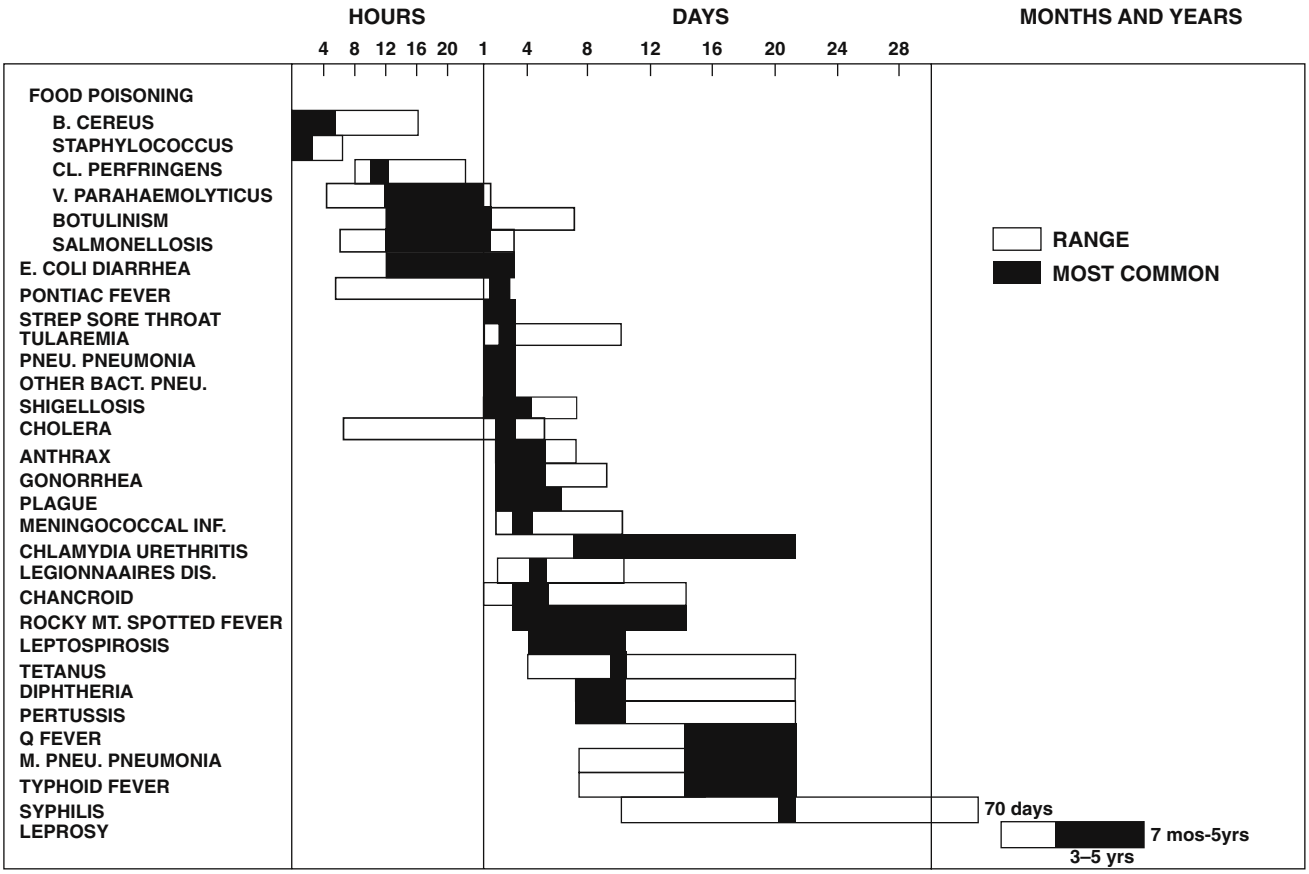


Figure 2. Incubation periods of common bacterial diseases. Derived from *Control of Communicable Diseases in Man*.[17]

infectiousness; (5) it provides a guide to the possible effectiveness of active or passive immunization; and (6) it gives clues to the pathogenesis of the disease.

The IPs of common bacterial diseases are given in Figure 2. Signs and symptoms due to preformed toxins or associated with food poisoning usually occur within 36 h after ingestion, sometimes as soon as 2–4 h, as in diarrhea due to *Bacillus cereus* or staphylococcal contamination of food. Traveler's diarrhea due to toxigenic *E. coli* has an IP of 12–72 h. Pontiac fever, the term used to describe an acute febrile disease without pneumonia recognized first in the Pontiac, Michigan, health department clinic and due to *L. pneumophila*, has a peak IP of 36 h, as compared to a peak IP of 5 days for Legionnaires' disease (pneumonia). It is not known whether this difference is due to a larger number of organisms inhaled in Pontiac fever (unlikely because of the comparative mildness of the disease), to dead organisms, or to some other factor.

Diseases due to direct involvement of epithelial surfaces have relatively short IPs, often under a week, such as streptococcal sore throat, bacterial pneumonias, shigellosis, cholera, gonorrhea, and chancroid. This is not invariably true, since diphtheria and pertussis both tend to have an IP of over a week, sometimes up to 3 weeks, and *M. pneumoniae* pneumonia has an IP of 2–3 weeks. These organisms may be less pathogenic. Diseases with longer incubation periods in the range of 2–3 weeks include systemic infections such as typhoid fever and brucellosis. The IP of syphilis most commonly is 3 weeks, although it may be as short as 10 days. Leprosy has an extremely long IP of 7 months to over 5 years.

10. Immune Response

A concomitant requirement for the evolution of multicellular life-forms was the development of methods for assuring the integrity of self. In the absence of the ability to recognize and cull out foreign cells, multicellularity could not have succeeded as a strategy. The mammalian immune

system is probably the most sophisticated antimicrobial defense in nature and represents the culmination of a billion-plus years of evolutionary refinement to the methodology of discriminating self from nonself.

Defense against microorganisms takes place at several levels in mammals. The term *innate immunity* refers to various features of an animal that confer natural resistance to invasion by microbes. These include physical barriers such as the skin and mucous membranes, chemical barriers (e.g., skin pH, lysozyme in saliva and tears), and the symbiotic bacterial flora of an animal that discourage colonization by pathogenic microbes.

Nonspecific immunity involves those processes in the immune response that do not react to specific individual foreign bodies and materials (antigens), but react to all foreign antigens with more or less equal vigor. The nonspecific immune system includes phagocytic cells, such as macrophages and polymorphonuclear neutrophils, the complement system, natural killer cells, cytokines such as the interferons and tumor necrosis factors, eosinophils, and basophils.

The third component of mammalian immunity is *antigen-specific immunity* or *adaptive immunity*. It is this component that appears to be unique among the vertebrates. Orchestrated by the lymphocytes, antigen-specific immunity recognizes foreign antigen in a highly specific fashion, expanding and activating only that subset of lymphocytes that can directly recognize and engage that precise antigen and no other. One of the most remarkable features of the antigen-specific immune system is the breadth of its capacity: The mature human immune system has been estimated to have the ability to respond discreetly to as many as a billion different antigens.

What follows is a very cursory overview of the components of the human immune system. For readers interested in more in-depth coverage, consult the general textbooks by Paul[46] or Kuby.[47]

10.1. Nonspecific Immunity

Nonspecific immunity plays numerous critical roles in the immune system. Many of the cells involved in nonspecific immunity participate in inflammatory responses and include macrophages, neutrophils, eosinophils, basophils, and mast cells. Overt tissue injury, whether caused by physical insult or invasion by a pathogen, stimulates the release of chemotactic factors that promote the migration and accumulation of various inflammatory cells at the damage site. Macrophages and neutrophils, particularly when activated by T-cell cytokines such as interferon-γ (IFN-γ), interleukin-4 (IL-4), and granulocyte-monocyte colony-stimulating factor (GM-CSF), are aggressively phagocytic cells. In the earliest phases of an infection, these cells serve to destroy bacteria and viruses and to mop up any debris, such as dead cells. Eosinophils are poorly phagocytic but are prominent in parasitic lesions, where they may be triggered to release a potent cocktail of enzymes and chemicals that destroy infecting parasites. Basophils and mast cells also have roles in immunity to parasites, but more as facilitators for the accumulation of other cells into a lesion.

Complement is a conglomeration of interacting proteins present in the bloodstreams of all vertebrates that have several important roles in nonspecific immunity. Various components of activated complement can promote and regulate inflammation, enhance phagocytosis through improved binding between the phagocyte and its target, and directly mediate cytotoxicity against cells and microbes. Several cleavage products of the complement activation cascade (C3a, C5a) have chemotactic and vasodilatory activity. A cleavage product of complement component C3, termed C3b, binds to specific receptors on macrophages and has lectin-like binding affinity for many bacteria, thus promoting opsonization. Complement activation culminates with the production of cannulus-like pores that self-insert into cell membranes (including those of bacteria), leading to colloid osmotic lysis.

Natural killer (NK) cells are a population of large granular lymphocytes that lack a receptor for specific antigen. They have a role in the immune surveillance of tumor cells and are capable of destroying their target cells by inducing apoptosis or programmed cell death.

Nonspecific immunity often serves to delay the progression of infection until the adaptive arms of the immune system have time to respond more specifically and effectively. Nonspecific immune mechanism is also frequently enhanced through the formation of partnerships with components of adaptive immunity. For example, specific antibodies may bind through specialized receptors to the surface of phagocytic cells, thus conferring antigen specificity to cells that otherwise have none. Cytokines secreted through the antigen-driven activation of T cells may enhance the level of activity of phagocytic cells or direct them to migrate to the site where they are needed. The activity of NK cells may also be enhanced by T-cell cytokines, particularly IL-2.

10.2. Humoral Immunity

There are two compartments of the immune system that are capable of responding specifically to foreign antigens. Humoral immunity, mediated by antibody molecules, is generated by B lymphocytes. Mature B lymphocytes bear specific receptors for antigen on their surface; the receptor on each circulating naive B lymphocyte is different from that

of all others, and each lymphocyte has one and only one specificity for an antigen. The receptor on the B lymphocyte is the immunoglobulin, or antibody, molecule itself, and upon activation, copies of this antibody are actively secreted. Since only those B lymphocytes that bind specifically with the antigens of a given bacterium or virus will be activated, the population of secreted antibodies that results is also specific for the invading pathogen.

With one limited exception, B-cell activation is absolutely dependent on the participation of T lymphocytes, which provide helper activity in the form of cytokines. T-cell cytokines are required for the activation of the B cell, for its differentiation into a plasma cell (the antibody-secreting cell), and for its proliferation (required to maximize the production of specific antibody). T-cell-derived cytokines also direct the class of antibody molecule that a B cell will ultimately produce.

Five distinct classes or *isotypes* of antibody molecules are found in the mammalian immune system, each of which has distinct roles in antimicrobial defense:

1. *IgM* has at least two important roles in humoral immunity. In monomeric form, it is expressed on the surface of all mature, naive B lymphocytes and serves as the receptor for antigen on those cells. In the circulation, IgM occurs as a pentamer, with five identical subunit antibodies bound together by a protein called J chain. IgM is the predominant antibody produced during the first exposure to an antigen. In serum, pentameric IgM has the highest binding avidity of any of the isotypes of immunoglobulin. It is extremely efficient at agglutinating bacteria and is an excellent opsonin, promoting phagocytosis by macrophages and neutrophils. In addition, IgM is the most effective antibody at activating complement.
2. *IgG* is the most abundant immunoglobulin in serum, making up 75% of the total serum antibody. It is the predominant antibody produced in a secondary or anamnestic immune response. Four distinct subclasses of IgG are recognized. All four subclasses of IgG can activate the complement cascade, although with widely different efficiencies. In addition, specific receptors for the constant region of all four IgG subclasses have been identified on a variety of cell types, including macrophages, mast cells, neutrophils, and lymphocytes. The combination of antibody with these other cell types either confers opsonic capacity or leads (in the presence of specific antigen) to the release of mediators. On lymphocytes, the binding of immunoglobulin molecules to such receptors appears to have regulatory functions, such as switching off the secretion of antibody. IgG is the only immunoglobulin that is capable of crossing the placenta, a process that is mediated by a specific receptor for IgG located on placental cells. In addition to its other activities, IgG is the most important neutralizing antibody in the serum, by virtue of its relative abundance.
3. *IgA* is the principal antibody found in local secretions. IgA antibody can be found in tears, saliva, milk, sweat, lungs, nasal passages, the urogenital tract, and the gut. IgA plays a crucial role in the defense of mucosal surfaces, neutralizing bacteria and viruses at local mucosa before they have an opportunity to invade. The presence of large quantities of IgA antibody in milk and colostrum enables the efficient transfer of maternal humoral immunity to newborn infants. IgA is incapable of fixing complement. It serves primarily as an agglutinating and neutralizing antibody.

   IgA in local secretions occurs as a dimer, bound by J chain. IgA transport to local mucosa is mediated by a molecule called the secretory piece, produced by cells adjacent to mucosal sites. IgA associates with the secretory piece on the cell surface and is endocytosed, transported across the cytoplasm, and deposited at local mucosa.
4. *IgE* makes up less than 0.01% of the total serum antibody. IgE is best recognized for its role in mediating immediate-type hypersensitivity, caused by the degranulation of mast cells and basophils in various mucosal tissues. IgE has an important role in the response against parasites. The purpose of the release of vasoactive substances when IgE bound to the surface of a mast cell or basophil encounters antigen is to enhance the accessibility of neutrophils and eosinophils, serum IgG, and complement to the site of parasitic infection.
5. *IgD* has no direct role in mediating defense against infectious diseases. It is present, together with monomeric IgM, on the surface of all naive B lymphocytes and appears to have a role in primary B lymphocyte activation.

10.3. Cell-Mediated Immunity

The second compartment of antigen-specific immunity is cell-mediated immunity (CMI). It is mediated by T lymphocytes. T cells are superficially indistinguishable from B cells. The T-cell receptor for antigen is structurally similar to the B-cell receptor, and yet the two cell types recognize specific antigen in fundamentally different ways. B cells recognize intact antigen and respond with the production of molecules that bear an exact copy of the surface receptor's binding site. T cells have no capacity for recognizing intact antigen. To engage a T-cell receptor, antigen first must be processed into small peptides, which are then inserted into the binding cleft of a major histo-compatibility (MHC) antigen. The MHC antigens are a highly polymorphic family of dual polypeptide proteins, at least some of which are expressed on nearly every cell type in the body. Because there are at least several hundred allelic variants of MHC antigens, and because no human being can express more than 12 different variants (up to two each of six different subclasses), the likelihood of two randomly selected individuals expressing the same panel of MHC antigens is remote in the extreme. Thus, the MHC antigens expressed by an individual to a very large extent define the property of self. This is particularly true for T cells. During the process of maturation in the thymus, nascent T cells undergo a rigorous selection process, and only cells displaying an antigen receptor that has a weak binding affinity for one or more self-MHC antigens are permitted to complete their differentiation into mature T cells. Cells with no affinity for self and cells with too strong an affinity, i.e., strong enough to activate a T cell on its own, are destroyed. When a foreign

peptide is inserted into the cleft of an MHC molecule, its affinity is altered. A small number of T cells, perhaps one in half a million, will bind to this altered MHC with a high enough affinity to activate. Thus, T cells are able to recognize and respond to minute changes in the normal display of self, as represented by an individual's unique collection of MHC molecules.

There are two general effector mechanisms for CMI. The first, cytotoxic T-cell-mediated lysis, is mediated primarily by the subset of T cells that express the protein CD8 on their surfaces. These T cells are restricted to their recognition of foreign peptide on the surface of a class I MHC molecule, which are present on most of the body's cells. Once activated, these cells recognize their specific combination of foreign peptide and MHC on a target cell and have two methods for dispatching the target. The first involves the deposition of perforin pores, which bear a striking resemblance to the terminal complement complex, onto the surface of the cell. The second approach is to induce apoptosis in the target, directing the cell to essentially commit suicide. Both processes result in the destruction of the target cell. Cytotoxic T lymphocytes (CTL), by virtue of their ability to discern the presence of foreign peptides from microbial pathogens inside of a cell, are ideally suited for the elimination of intracellular parasites such as viruses. They are also considered to have an important role in the elimination of tumor cells.

Another CMI mechanism is mediated primarily by the other main subpopulation of T cells, those that express the protein CD4 on their surface. These T cells are restricted in their recognition of foreign peptides on class II MHC molecules. Class II MHC antigens are normally expressed on only a few populations of cells in the body, chiefly B cells, macrophages, and dendritic cells. All of these cell types are capable antigen-presenting cells, since $CD4^+$ T cells also have the central role in the regulation of T-cell responses; the limited expression of class II antigens in the body is thought to reflect a safety restraint aimed at limiting the responsiveness of immune cells. In addition to providing helper activity to both B and T cells, $CD4^+$ T cells are typically recruited to the site of tissue damage and infection, where they coordinate and amplify the effectiveness of both specific and nonspecific arms of the immune system.

10.4. Cellular Interaction in Immune Responses

The immune system is arrayed with an arsenal of weapons capable of causing colossal destruction of tissues if left unchecked. While it provides an invaluable service in the defense against microbial invasion and the spontaneous development of neoplastic cells, extremely tight controls are required to ensure that its activities do not escalate to the point where they become deleterious to the host. As such, the level of intercellular interaction in the immune system is extraordinarily high. As mentioned previously, B-cell responses are generally not possible in the absence of T-cell help. If specific cytokines, such as IL-2 for proliferation, are not made available to the antigen-stimulated B cell, a suboptimal response will result. The generation of T-cell help, in turn, is absolutely dependent on the presence of a suitable antigen-presenting-cell-bearing peptide on a class II MHC antigen. Thus, the participation of at least three different cell types is required to mount an effective immunoglobulin response to antigen. Similarly, a CTL response requires the participation of both a helper T cell and an antigen-presenting T cell for the activation of both T-cell help and the naive CTL.

These cellular interactions take place in at least two ways. Transmembrane surface proteins on both participating cell types may come into direct physical contact, potentially sending an activation signal across the cytoplasmic membrane into both cells. An alternative unidirectional signaling method is to produce soluble hormone-like proteins known as cytokines that bind to a specific ligand on their target cell and send an activation signal across the membrane. A large number of cytokines have been identified and characterized, and each cytokine typically has more than one activity, often depending on the type of cell it engages.

Cellular interactions are not limited to the activation of immune responses. When a threat to bodily integrity has been eliminated, there is no continued need for a response. The action of down-regulating immune responses is termed *immune suppression*. T cells are also the critical regulators for immune suppression. Many of these functions of immune regulation appear to be performed by discreet subpopulations of specialized T cells. At last count, there are at least several functionally distinct subsets of helper T cells, a similar number of suppressor T cells, and so forth. In addition, functionality among T cells does not appear to be set in stone. There are circumstances in which $CD4^+$ T cells perform cytotoxic function and population of $CD8^+$ T cells that serve various regulatory functions. Thus, the complexity of interaction among cells in the human immune system is quite high, and a meaningful discussion of the current, admittedly limited, level of understanding of those interactions is beyond the scope of this overview. Suffice it to say that, under ordinary circumstances, these interactions serve as a system of controls that ensure the immune system will mount a transient, surgical strike against any disease threat.

Occasionally, elements of the interregulatory network break down, sometimes leading to autoimmune disease, a condition in which the immune system causes damage to

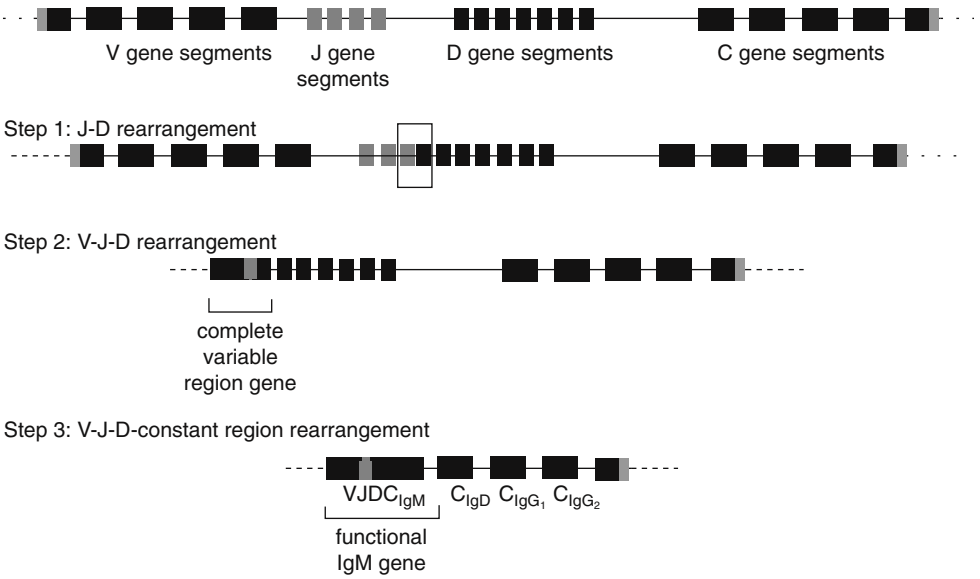


Figure 3. Immunoglobulin heavy chain gene cluster.

various host tissues. Such diseases include type I diabetes, rheumatoid arthritis, and systemic lupus erythematosus. Autoimmune diseases run the gamut from chronic disabling conditions to life-threatening diseases.

10.5. The Generation of Diversity in Antigen Recognition

The immune system presents a great enigma that was a matter of pure philosophical speculation until only two decades ago, namely how does the body go about the business of preparing a population of cells, each of which bears a distinct receptor for one of as many as a billion different antigens, none of which it has ever seen before? The two extremes of the philosophical argument went as follows: (1) One's genetic material contains the information required to encode all one billion different specificities for foreign antigen, and selects from that collection of genes in a random fashion; and (2) the genetic material encodes for only a few antigen receptor genes and the rest of the diversity results from extraordinarily high rates of mutation. The interesting feature of these two "obvious" solutions to the enigma is that neither of them came terribly close to the truth.

Immunoglobulin genes and T-cell receptor genes are arranged in clusters of subsegments, as shown in the illustration of an immunoglobulin heavy chain gene map (Figure 3). The immunoglobulin genes and T-cell receptor genes are assembled from randomly selected segments into a single functional gene. The construction of variable region (which encodes the antigen binding site) of a heavy chain gene, for example, would involve the co-alignment of one of approximately 1,000 V gene segments, with one of four J-segment genes and one of 12 D-segment genes. This results in $1{,}000 \times 4 \times 12 = 48{,}000$ possible heavy chain variable gene segments. The various segments are aligned through intrachain recombination events. Recombinational diversity for the immunoglobulin light chains is somewhat more limited, providing for approximately 1,000 different light chain variable genes. Since every functional antibody molecule is made up of two identical light chains and two identical heavy chains, the number of different combinations becomes $1{,}000 \times 48{,}000$ or 480,000. Added to that is the fact that the junction sites between V, J, and D segments vary, leading to junctional diversity due to single base substitutions and even short frame shifts. Finally, the mutation rate in the immunoglobulin gene cluster is approximately fivefold that of normal mutation rates. All of this is estimated to provide potential for diversity in the antibody population on the order of a billion different specificities. The T-cell receptors rearrange similarly.

11. Patterns of Host Response

The clinician usually deals with persons already ill with an infectious disease severe enough for them to seek medical care. The epidemiologist must study not only those clinically ill but also the full range of host responses that follow infection. These can vary quantitatively from inapparent infection

to severe illness to death in what is called a *biological gradient*. They can also vary qualitatively in different signs and symptoms that make up different clinical syndromes. The detection of persons with inapparent or subclinical infection requires laboratory tests to detect the presence of antibody or antigen.

11.1. Biological Gradient

When a susceptible host is exposed to a source of infection, a wide range of quantitative responses may occur.[4] These are often depicted as an iceberg, as shown in Figure 4, with the largest number of responses occurring subclinically, below the waterline of clinical recognition. The right side of Figure 4 represents the responses of the host. These range subclinically from exposure without successful attachment or multiplication of the bacterial organism, to colonization without tissue injury, to infection that evokes a host immune response but no clinical disease. The existence of these inapparent events can be recognized only by laboratory means such as isolation of the organism or measurement of the immune response. In viral infections, the ratio of inapparent–apparent (subclinical–clinical) infection has been determined through prospective studies correlating the number with an antibody response to the number clinically ill. The occurrence of inapparent infections is also suggested in persons with antibody but no history of clinical disease. Some information has also come from the rate of secondary infection in families with an index case and from volunteer studies. This type of information is not available for many bacterial infections because serological techniques are not as useful and/or widely employed in measuring infection rates, but a few examples may be cited. In leptospirosis, serological tests of persons heavily exposed (veterinarians, abattoir workers) but without known illness have shown an antibody prevalence of 16%. This suggests that only 16 of 100 exposed persons have been infected as manifested by an antibody response. There are no data for leptospirosis indicating the subclinical–clinical ratio, but of those clinically ill, 90% or so have an acute, self-limited illness without jaundice and with good prognosis; the overall case-fatality rate in 791 cases reported to the CDC from 1965 to 1974 was 7.7% (see Chapter 20). In tuberculosis, it was estimated that of the 50,000 clinical cases reported in 1963, 80% came from the 24 million infected in previous years and 20% from persons infected the same year (see Chapter 39).

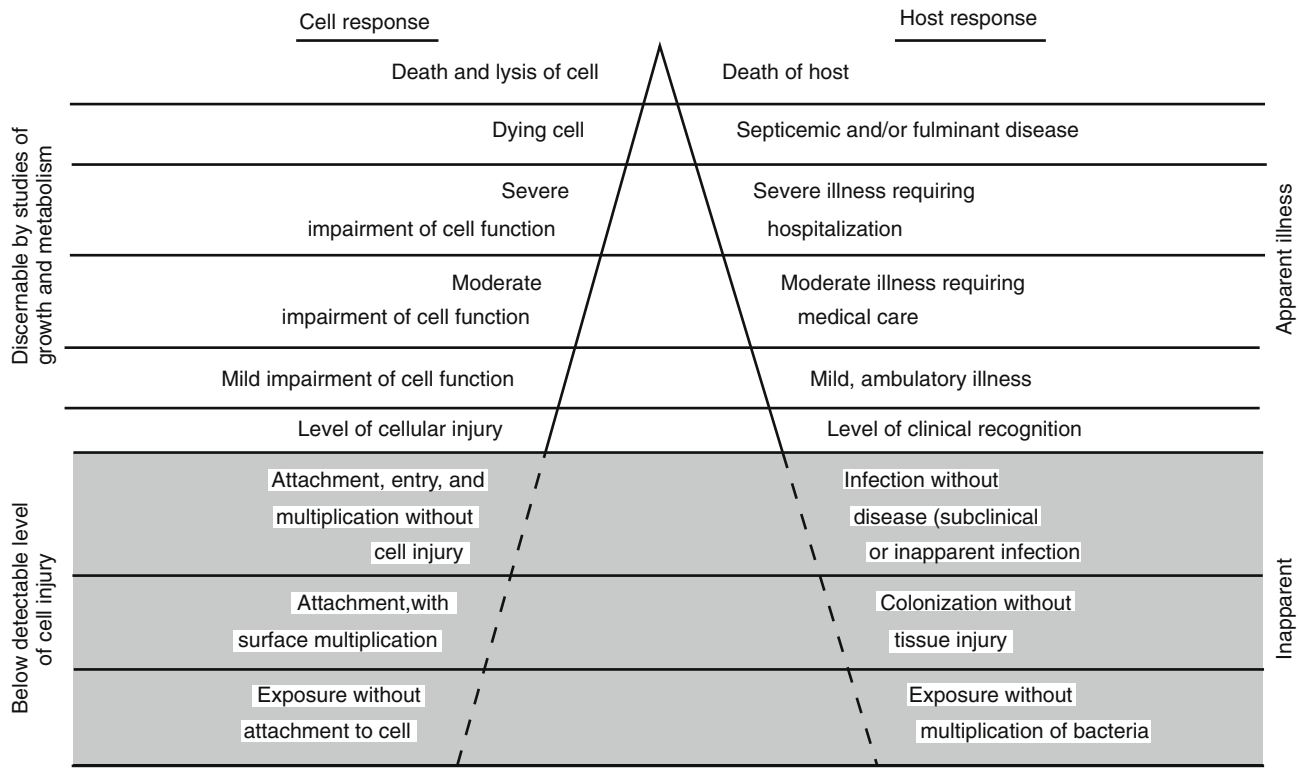


Figure 4. Biological spectrum of response to bacterial infection at the cellular level (*left*) and of the intact host (*right*).

In summary, a wide range of quantitative and qualitative responses can occur on exposure to a pathogenic organism. The determinants of this pattern lie both in the pathogenicity and virulence of the infecting organism (see Section 4) and in the age, genetic makeup, immune response, portal of entry, and other characteristics of the host (see Section 6).

The left side of Figure 4 is a simplistic expression of the response to bacterial infection at the cellular level. Cell injury may result from enzymes and other metabolic products of bacteria, from toxins produced by them, from entry and multiplication of the organisms intracellularly, as with *M. tuberculosis, M. leprae, B. abortus*, or as a consequence of the phagocytic and immunologic defense mechanisms induced by the infection. Epidemiologically, the message is that most organisms do not result in cell dysfunction or death and that the healthy person lives in symbiosis with millions of bacteria.

11.2. Clinical Syndromes

The host can show qualitative as well as quantitative differences in response to the same bacterial infection. These qualitative responses are manifested by different clinical syndromes, the patterns of which depend on the portal of entry of the organism, age of the host at the time of infection, immune status, and other factors. This variation is not as great with bacterial infections as with viral infections. Many bacterial infections such as anthrax, cholera, diphtheria, leprosy, pertussis, tetanus, tularemia, and typhoid fever present with fairly characteristic clinical features that vary quantitatively, but not qualitatively, from host to host, and a diagnosis can often be made on clinical grounds alone. Others, such as streptococcal infections, leptospirosis, syphilis, tuberculosis, and Lyme disease, may involve different tissues and organs in different persons, resulting in different clinical presentations, often depending on the site of infection and the age group involved. On the other hand, there are many clinical syndromes of diverse cause in which etiologic diagnosis is difficult on clinical grounds alone. It is here that epidemiological probabilities will help clinical judgment. Infections of mucosal and serosal surfaces fall into this category because of the limited spectrum of local responses that can result. These can involve the meninges, respiratory tract, intestinal tract, urinary system, and urethra. Invasion of the bloodstream (septicemia) by different bacteria also invokes a common group of signs and symptoms that may be difficult to differentiate etiologically.

This section will present some of the common causes of these clinical syndromes that may vary with the age of the person at the time of infection. Knowing the most common causes in a given age group may help to establish a tentative diagnosis and to initiate proper treatment.

11.2.1. Infections of the CNS. Meningitis is a common clinical syndrome caused by bacteria, viruses, fungi, and protozoa. The clues that suggest bacterial infections are polymorphonuclear leukocytosis in blood and cerebrospinal fluid (CSF) (500–20,000/mm^3), low CSF glucose concentrations (usually $<$35 mg/100 ml or CSF–serum ratio$\leq$0.5), elevated CSF protein (80–500 mg/100 ml), and in about 75%, the presence of bacteria in the Gram-stained smear of a centrifuged sample of CSF. These findings characterize purulent meningitis, but may be altered by treatment with antibiotics so that they resemble nonpurulent bacterial infections, such as *M. tuberculosis* and leptospirosis, or viral meningitis. The etiologic agents that produce meningitis in different age groups will vary some by geographic area, year, and socioeconomic level.

The age-specific incidence per 100,000 of various types of meningitis by age group is presented in Table 9 from an excellent review article on the diagnosis and management

Table 9. Annual Age-Specific Incidence of Meningitis, United States, 1978–1981[a]

Age	*Neisseria meningitidis*	*Haemophilus influenzae*	*Streptococcus pneumoniae*	Group B *streptococcus*	*Listeria monocytogenes*	Total meningitis
$<$1 mo	2.0	6.7	3.5	44.6	7.6	99.5
1–2 mo	9.1	18.6	5.7	10.0	1.3	56.7
3–5 mo	11.5	52.0	11.6	1.4	0.1	83.3
6–8 mo	10.6	65.1	8.0	0.3	0	88.4
9–11 mo	7.9	48.1	4.7	0	0.1	63.3
1–2 yr	3.8	19.0	1.5			25.3
3–5 yr	1.8	3.9	0.5			6.8
5–9 yr	0.7	0.7	0.3			2.0
10–19 yr	0.6	0.1	0.1			1.0

[a]Results are reported as numbers of children with meningitis per 100,000 population.[48] Data were provided by C. V. Broome, Centers for Disease Control, Atlanta, and by Schlech et al.[49]

of meningitis by Klein et al.,[48] which incorporates data from the CDC and a National Surveillance Study by Schlech et al.[49] The overall incidence of meningitis is highest in the newborn, drops in the first 2 months of life, and then rises to high levels between 3 and 8 months of life. Based on a National Surveillance Study of 18,642 cases reported from 1973 to 1981, *H. influenzae* accounted overall for 48.3% of the cases, *N. meningitis* for 19.3%, and *Streptococcus pneumoniae* for 13.3%, totaling 81%.[49] Rates in males exceeded females (3.3 vs. 2.6 per 100,000). By age, the most common causes were as follows: in the newborn, group B streptococcus and *E. coli*; in infants, *H. influenzae* type b and *N. meningitidis*; in toddlers, similar to the newborn; and in schoolchildren and adolescents, *S. pneumoniae, N. meningitidis*, and *H. influenzae* are the major pathogens in the decreased number of cases in that age group. An analysis of 493 episodes in 1993 of bacterial meningitis in hospitalized adults over 16 years of age in a Boston hospital from 1962 to 1988 revealed that 40% were of nosocomial origin with a mortality of 35%.[50] The risk factors included age $\geq$ 60 years, obtunded mental state on admission, and seizures within the first 24 h. Gram-negative bacilli (other than *H. influenzae*) caused 33% of these episodes. Of 296 community-acquired meningitis cases, *S. pneumoniae* accounted for 37%, *N. meningitides* for 13%, and *L. monocytogenes* for 10%. Only 4% of all episodes were due to *H. influenzae*.[50]

The characteristics of the organisms involved and the patterns of disease can be found in the individual chapters of this book. Suffice it to mention here that in the newborn, most cases of group B streptococci are due to subtype III, and that infection usually arises from vaginal and rectal infections of the mother that can lead to a 40–70% infection rate in the newborn; the K1 antigen of *E. coli* is involved in 75% of neonatal meningitis, and *L. monocytogenes* is becoming increasingly important in meningitis in this age group in several areas of the country.[48] In children beyond the newborn period, head trauma may precede the onset of meningitis and organisms may then enter through the cribiform plate or the paranasal sinuses. Meningitis may also follow neurosurgical procedures or osteomyelitis of the skull or vertebral column.

Acute bacterial meningitis is a medical emergency, and it is most important to establish the identity of the infecting organisms as quickly as possible as a guide to antibiotic therapy. However, until this is done, a Gram-stained smear of the CSF should provide a reasonable basis for initial chemotherapy.

11.2.2. Acute Respiratory Infections. These represent the commonest causes of morbidity in the developed world. Their importance as a cause of both morbidity and mortality in developing countries has now been recognized, and major programs of control have been initiated under the auspices of the WHO. In developing countries, it is estimated that over 4.5 million children under the age of 5 years die annually of acute respiratory tract infections (ARI), which represents some 30% of the 14 million deaths in this age group yearly.[51–54] The commonest causes are *S. pneumoniae* (see Chapter 34) and *H. influenzae* (see Chapter 16). A study of respiratory infections in children aged 0–59 months in several different developing countries revealed rates of 12.7–16.8 new episodes of ARI per 100 child-weeks at risk and 0.2–3.4 for lower respiratory tract infections.[55] Case management intervention strategies using community health workers have helped reduce the mortality, but many gaps remain in our understanding of the etiology, epidemiology, and pathogenesis of these conditions.

Fewer than 10% of acute upper respiratory infections (AURIs) are due to bacteria in either developed or developing countries, although in the latter, infections with *B. pertussis* (see Chapter 26) and *M. pneumoniae* (see Chapter 24) are important in certain settings. The syndrome of epiglottis in children aged 6 months to 2 years (up to age 6), however, is due to a bacterial pathogen, *H. influenzae* type b in about 90% of cases worldwide. This is often a serious and fulminant infection with a high mortality. A vaccine is now available.

The viruses involved in AURIs in both developing and developed countries are respiratory syncytial virus (RSV) in children under 3 years of age, parainfluenza in older children, and coronaviruses, rhinoviruses, and influenza in all ages.

The syndrome of acute pharyngitis and tonsillitis is due to streptococcal infections, mostly group A, in about one fourth to one third of cases, another one third are due to various viruses, and the remainder are of unidentified cause, although chlamydial infections may play a role in some of these.[56,57] Streptococcal infections can lead to acute rheumatic fever; this disease had been disappearing rapidly in developed countries until very recently when a recrudescence was reported in several areas of the United States. A very invasive streptococcal group A infection producing a toxic shock-like syndrome has recently been described in several parts of the world[58,59] (see Chapter 38). In developing countries, some 1.2 episodes of rheumatic fever are said to occur for every 1,000 untreated streptococcal infections, but this, too, seems to be decreasing.[52] *C. diphtheriae* (see Chapter 13) is also a cause of exudative tonsillitis in some Third World countries, and a recent outbreak occurred in Sweden involving 17 cases and 3 deaths despite very high immunization coverage.[60]

Acute lower respiratory infections are due mostly to viruses in young children, except for infants, to

M. pneumoniae and viruses in young adults, and to bacterial pathogens in older adults and the elderly. Prospective studies in community settings of developed countries suggest that five agents—RSV, parainfluenza virus, influenza virus, adenovirus, and *M. pneumoniae*—account for some 80% of acute lower respiratory infections in these population groups.[52]

Common respiratory syndromes in young children such as croup, laryngotracheitis, and bronchiolitis are usually due to viruses, especially RSV and parainfluenza viruses. Diphtheria may also cause a croup syndrome when the toxic membrane involves the larynx. Acute bronchitis is most often due to a variety of viral infections, but bacteria such as *M. pneumoniae, B. pertussis*, and *C. trachomatis* (TWAR strain) may also produce this syndrome. Chronic bronchitis, on the other hand, is largely associated with nontypable *H. influenzae* and pneumococci (*S. pneumoniae*).

Pneumonia is the sixth most common cause of death in the United States and has varied causes at varied ages. In infancy (under 6 months), about 30% are now recognized as chlamydial and present as a gradually developing nontoxic illness with cough, pulmonary congestion, rales, and patchy infiltrates on X-ray (see Chapter 10). Chlamydial genital infection of the mother carries a 10–20% risk of pneumonia in her infant. In another study of 205 infants under 3 months old hospitalized with pneumonitis, Brasfield et al.[61] identified a causal agent in 70%. *C. trachomatis* was found in 36%, RSV in 23%, cytomegalovirus in 20%, *Pneumocystis carinii* in 17%, and *Ureaplasma urealyticum* in 16%. In older children, the causes of pneumonitis and pneumonia are roughly one fourth viral, one third unknown cause, and the rest bacterial. More careful bacteriological culturing techniques have identified a bacterial etiology in 15–50% of this age group: pneumococci (*S. pneumoniae*) are the most common organism, and *H. influenzae* next most common; rarely, *S. aureus* and group A streptococci are involved.

A review of the bacteriological aspirates in children with pneumonia from many countries revealed a bacterial agent in 62% of 1,029 cases: *H. influenzae* and *S. pneumoniae* accounted for 54% of all isolates, while *S. aureus* was responsible for 17%.[52] Viral infections were associated with 17–40% of pneumonia in hospitalized children in several developing countries.[52] The true role of *Chlamydia* and *M. pneumoniae* infections in childhood respiratory infections in developing countries has not been well studied yet because of technical problems with the laboratory diagnosis, but it seems likely they may play as important a role as in developed countries. Community studies of *M. pneumoniae* pneumonia have shown the highest incidence in school-age children, with a decline after puberty, and with the lowest rates in the over 40-year-old age group, possible reflecting immunity. However, some 25–50% of pneumonia in young adults, such as college students, is due to this organism, and infection rates are high in military recruits (see Chapter 24). In a long-term study of some 15,000 episodes of pneumonia in a group health plan in Seattle, about 45% were tested for *M. pneumoniae* by isolation and serological tests, and 15% were found to be due to this agent, with the highest rate in children.[62] A new chlamydial strain, designated as TWAR (for Taiwan acute respiratory), has been shown to be an important pathogen in acute respiratory infections such as pneumonia, bronchitis, sinusitis, as well as mild upper respiratory infections.[63,64] In a retrospective examination of 200 patients from a group cooperative study,[62] 8% were due to this organism, which is a rate of 1 per 1,000 per year and with the highest incidence in the elderly. Only about 2% of patients with *M. pneumoniae* require hospitalization, and infection with *Chlamydia pneumoniae* is usually mild or asymptomatic. The secondary intrafamilial infection rate is high after an index case of *M. pneumoniae* is introduced and involves about 84% of children and 41% of adults.

In adults, especially older adults, most pneumonias are bacterial in origin. In the past, *S. pneumoniae* caused 50–90% of community-acquired pneumonias, but in recent years this has decreased to some 16–60%, still the dominant organism.[65–67] In an excellent prospective study of 359 community-acquired cases of pneumonia admitted to three Pittsburgh hospitals in 1986–1987, the most frequent etiologic agents were *S. pneumoniae* (15.3%), *H. influenzae* (10.9%), *Legionella* spp. (6.7%), *C. pneumoniae* (TWAR) (6.1%), and gram-negative organisms (6%).[65] In 32.9%, the etiology was indeterminate. No distinctive clinical picture was diagnostic for any etiologic agent. The underlying illnesses were immunosuppression (36.3%), chronic obstructive pulmonary disease (32.4%), and malignancy (28.4%). The median age was 62 years. The mortality was 13.7% and was highest for *S. aureus*. For most cases, erythromycin therapy would be effective. In recent years, gram-negative organisms are of increasing importance, perhaps causing 7–18% of pneumonia, and of special importance in the elderly. Some 30–40% of community-acquired pneumonias are of unknown etiology, due perhaps to 30% of persons unable to produce sputum for culture, or one third who have received antibiotics before hospitalization, or in about one fourth the organisms are difficult to isolate, such as *M. pneumoniae, C. pneumoniae, Coxiella burnetti*, and *Legionella* species, as well as viruses. Even invasive diagnostic techniques in three studies left 43% of unknown etiology, 8% were polymicrobial, and 49% were due to a wide variety of pathogens that varied according to geographic region.[67] Antibiotic-resistant organisms and toxic streptococcus are emerging pathogens in pneumonias, and *M. pneumoniae* is appearing as a pathogen in the elderly.[68]

11.2.3. Acute Otitis Media. This is a common infection of children, with a peak age of 6–18 months. It occurs seasonally throughout the year, but is highest in fall and winter.

Family aggregation is common for recurrent disease. Accurate diagnosis is dependent on cultures taken by aspiration from the middle ear. A recent review of etiology by Klein[69] reports that a bacterial antigen was isolated from two thirds of such aspirations: *S. pneumoniae* was the leading cause in 25–50%, then nontypable *H. influenzae* in 15–30%, *Moraxella catarrhalis* in 3–20%, group A streptococci and staphylococcus in 2–3%, and viruses alone or with bacteria in about 33%. *M. pneumoniae* was uncommon in middle-ear infections in most children. In children under 6 months, *C. trachomatis* with acute respiratory infections was found to be associated with middle-ear infections. It is important to emphasize that the etiology cannot be diagnosed by the clinical picture alone and that culture of aspirated material from the middle ear is needed.

11.2.4. Intestinal Infections and Intoxications. These may be divided into foodborne poisons and intestinal infections.

11.2.4.1. Bacterial Food Poisoning. While this section is concerned with bacterial causes, it is important to emphasize that Norwalk and Norwalk-like viruses are probably one of the major causes of both food- and waterborne outbreaks worldwide.[70] Bacterial food poisoning is dealt with in more detail in Chapter 5. In an analysis of 2,841 outbreaks of known etiology from 1973 to 1987 reported to the CDC and which involved 124,824 persons, 66% were caused by bacterial pathogens, 25% were due to chemicals, 5% to parasites, and 5% to viruses. The major contribution of bacterial etiology to the 124,824 cases was *Salmonella* (45% of total), involving 55,864 cases in 790 outbreaks; *S. aureus* (14% of total), involving 17,248 cases in 367 outbreaks; *Shigella* (12% of total), involving 14,399 cases in 104 outbreaks; and *C. perfringens* (10% of total), with 12,234 cases in 190 outbreaks. *C. botulinum* was reported in 231 outbreaks (8%) and affected 494 persons.[71] It should be emphasized that food outbreaks are greatly underreported.

11.2.4.2. Intestinal Infections. The causes of enteric infections vary with age and geographic area. They are perhaps the most common cause of morbidity worldwide, rivaling acute respiratory infections. In this book, they are dealt with under *Campylobacter* (Chapter 8), cholera (Chapter 11), *C. difficile* (Chapter 12), *E. coli* (Chapter 14), *Helicobacter pylori* (Chapter 17), salmonellosis (Chapter 31), shigellosis (Chapter 32), typhoid fever (Chapter 42), and yersiniosis (Chapter 43).

The magnitude, special settings, and etiology of diarrhea in both developing and developed countries have recently been reviewed.[72] The incidence in developed countries is estimated as 1–3 illnesses per person per year and 5–18 illnesses per child per year in tropical, developing countries. An estimated 12,600 deaths per day occur in children in some of these developing areas of Asia, Africa, and Latin America. In all countries the peak incidence is in young children, usually under age 3 years and often earlier in developing countries. The causes involve bacteria, viruses, and parasites, and sophisticated laboratory techniques are required to diagnose some of them, thus making definitions of etiologic patterns difficult in areas without modern laboratory facilities.

In children, viruses play a predominant role in some seasons, and the rotaviruses may cause about half the cases of acute diarrhea in children under age 3 years on a worldwide basis. In older children and adults, Norwalk-like agents are important, and intestinal adenoviruses are increasingly being recognized in young children. Among the bacterial pathogens, enterotoxic *E. coli* is probably the most common, with its production of both the cholera-like LT toxin and the ST toxin.[72]

In travelers, *E. coli* (ETEC) is also the most common cause and is found in 42% of diarrheal episodes in Latin America, 36% in Africa, and 16% in Asia.[42] The enterotoxigenic form has been found in 15–30% of illnesses in the community or in hospitals, especially in tropical, developing settings, and is also common in adults in these areas.[72] *C. jejuni* is perhaps the most common cause of inflammatory diarrhea in developed countries and *H. pylori* is increasingly recognized as a cause of gastritis, peptic ulcer disease, and probably gastric carcinoma.[73,74] The organism can be eliminated in 80–90% of infections by treatment with bismuth and antibiotics, and such therapy is routinely recommended for gastric ulcers.[75]

Diarrhea is increased in incidence in certain special settings. The importance of nosocomial diarrhea is being increasingly recognized. It accounted for 17% of all nosocomial infections in a children's hospital in Buffalo[76] and 42% in an adult intensive care unit in a London hospital.[77] The etiology of nosocomial diarrhea as reported to the CDC from 1980 to 1984 was *C. difficile* in 45.1% and *Salmonella* species in 11.8%.[72] In extended care facilities for the elderly, diarrhea is also an important problem, where about one third of patients experience a significant episode each year, and *C. difficile* is a common cause. Outbreaks of diarrhea in child care centers are also common, and frequent causes include rotavirus, *Shigella, C. jejuni, C. difficile, G. lamblia*, and *Cryptosporidium*. High attack rates are seen with rotavirus and some bacterial infections. Finally, some 50–60% of patients infected with HIV in the United States have diarrhea at the onset of illness, and initial

diarrhea is seen in over 90% of AIDS patients in Africa and Haiti.[78] A wide variety of organisms may be involved, of which *Cryptosporidium* is the most frequent, accounting for 3–21% in AIDS patients in the United States and 50% in Africa and Haiti.[78] This organism may also produce diarrhea in normal persons and its contamination of a public water supply in Milwaukee resulted in a massive outbreak involving over 403,000 persons.[28]

As mentioned above, *Campylobacter* enteritis is an increasingly recognized cause of intestinal infections in the United States, Great Britain, and Australia, accounting for some 5–14% of the cases in several large series (see Chapter 8). The clinical disease is characterized by watery diarrhea with mucus, blood, or pus, often cramping abdominal pain and fever, and sometimes gross blood in the stools of children. *Y. enterocolitica* is another recently recognized cause of acute diarrhea attended by some cramps, fever, and occasionally a rash; the incubation period is 1–3 days. There may be associated mesenteric adenitis, arthritis, and erythema (see Chapter 43).

The prevention of traveler's diarrhea is important because some 40% of travelers from developed countries with temperate climates will develop diarrhea, as reviewed by DuPont and associates.[43,79,80] The use of bismuth subsalicylate has proved useful in the short term and antibiotic prophylaxis and treatment is recommended for longer exposures. The following recommendations are quoted from DuPont et al.[79]:

> In most cases a bacterial pathogen is responsible for the illness. The antimicrobial agents with the greatest activity against these organisms are cotrimoxazole (trimethoprim/sulfamethoxazole) during the summer months in the interior of Mexico (a region where this agent has been studied extensively), and the fluoroquinolones for other places or other times, until data become available to indicate the appropriateness of cotrimoxazole here as well. Persons at risk should take along with them a drug to treat symptoms of travelers' diarrhea, and an appropriate antimicrobial agent. At the passage of the third unformed stool, it is recommended that travelers treat themselves with fluids and salt (flavored mineral water augmented with saltine crackers is sufficient in most cases), symptomatic treatment and antibacterial therapy. Of these, the antimicrobial is the most important component, which is given either as a single large dose or once or twice daily for 3 days. Perhaps optimal therapy for afebrile nondysenteric patients is loperamide in combination with the antibacterial drug. In the face of fever or dysentery, the antimicrobial should be used alone. In special situations where food and beverage restrictions cannot be followed and where the itinerary cannot tolerate even the slightest alterations because of illness, chemoprophylaxis can be considered. The most effective preventive medication in this case is the antimicrobial also used for therapy, taken in half the therapeutic dosage daily while in the area of risk. However, the majority of travelers should not use this approach.

11.2.5. Acute Urinary Tract Infections. In various studies of acute urinary tract infections (UTI) of inpatients and outpatients, *E. coli* is by far the most common organism in both settings, accounting for up to 80% of initial urinary infections in outpatients.[81] In a more recent compilation of etiologic agents in hospitalized cases, Andriole[82] found 35.8% due to *E. coli*, 16.4% due to *P. mirabili*, 16.3% due to *Enterobacter aerogenes*, 10.1% due to *K. pneumoniae*, and less than 6% each due to other causes. Over 80% are associated with use of urethral catheters. The spectrum of urinary infections varies and each syndrome has its own unique epidemiology, natural history, and clinical manifestations.[83] The major risk factors include the newborn (particularly the premature), prepubertal girls, young boys, sexually active young women, elderly males, and elderly females. Risk factors that contribute to lower tract infection in women include sexual intercourse, diaphragm–spermicide use, and voiding behavior.[84] UTI is the most common infectious disease of the elderly and is especially prevalent in debilitated, institutionalized older individuals. Unlike UTI in younger women, which tends to be related to frequency of sexual intercourse and is uncomplicated, in the elderly it is more difficult to treat and its pathogenesis is related to abnormal bladder function, bladder outlet obstruction, vaginal and urethral atrophy, use of long-term indwelling catheters, and puddling related to bed rest. The spectrum of organisms causing infection relates to the ecology of the patients' environments; those residing in nursing homes, and especially with permanent indwelling catheters, tend to have a greater variety of pathogenic organisms, many of which may be relatively antibiotic resistant.[85]

The immediate diagnosis of UTI is based on relatively simple techniques. The presence of pyuria and bacteriuria, the two most important indicators of UTIs, is most accurately determined by standard techniques. In quantitating pyuria, the finding of 10 leukocytes/mm^3 of urine by either hemocytometry or direct microscopy correlates highly with symptomatic, culture-proven UTIs.[86]

11.2.6. Sexually Transmitted Diseases. The term *sexually transmitted diseases* (STD) encompasses the five classic venereal diseases (gonorrhea, syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale), plus other major ones such as HIV-1, HIV-2, human T-cell lymphotropic virus type I (HTLV-I), herpes simplex 1 and 2, papillomaviruses, hepatitis A and B, *C. trachomatis, Candida albicans*, and *Trichomonas vaginalis*. The number of cases and rates per 100,000 of major bacterial causes of STDs reported to state health departments in 1988 and 1992 are given in Table 10. While *C. trachomatis* infections are among the most prevalent of all sexually transmitted diseases, problems in diagnosis, lack of public health laws, and other problems have made surveillance of this disease

Table 10. Reported Cases and Rates per 100,000 of Classical Sexually Transmitted Diseases Reported to State Health Departments in the Civilian Population, United States—1988 and 1992[a]

	Number of cases		Rate per 100,000	
Disease	1988	1992	1988	1992
Gonorrhea	738,160	501,409	300.3	201.6
Syphilis (all stages)	104,546	112,541	45.5	45.3
Chancroid	4,891	1,886	2.0	0.8
Lymphogranuloma venereum	194	302	0.1	0.1
Granuloma inguinale	11	6	0.0	0.0

[a]Derived from Table 1, in Ref. 87.

incomplete, and therefore it is not included in Table 10. Reflecting increasing recognition and reporting of this infection, the rates per 100,000 have risen from 3.2 in 1984 to 182.6 in 1992 when 36 states reported to the CDC.[87] In large cities with more than 200,000 population the rates were 296.8 in 1992. An estimated 4 million cases occur annually, many of which cause pelvic inflammatory disease with serious sequelae such as ectopic pregnancy and infertility.

It is important to emphasize that many of these STDs occur in synergy with HIV infection and that the transmission of both is enhanced in the presence of the other. Overall, the trends in recent years show that reported rates for gonorrhea have declined 19%, with rates decreasing from 250 per 100,000 in 1991 to 202 per 100,000 in 1992, and with declines in primary and secondary syphilis rates from 17.3 to 13.7 in the same period. This decline may reflect the adoption of safer sexual practices. The proportion of adolescent women aged 15–19 years who reported having sexual intercourse, however, increased from 29% in 1970 to 52% in 1988.[88] The rates of gonorrhea and syphilis in the adolescent age group are three times that of the general population, and thus are a major target for education and control. The risk for STD is also much higher in blacks than in whites, with rates for primary and secondary syphilis being 60-fold higher and for gonorrhea 40-fold higher; for Hispanics the increases are fivefold and threefold higher, respectively.

Adequate surveillance data are not available for several sexually transmitted diseases. Some information of trends is seen in the office practice of certain physicians based on the National Disease and Therapeutic Index as analyzed in graphs in a CDC report.[87] For human papillomavirus genital warts, physician visits in this survey have risen from about 45,000 in 1966 to 200,000 in 1992. Trichomonal infections have declined slowly over this period, starting with 500,000 in 1966, but other vaginal infections have increased sharply from about 120,000 to 3,000,000.

Many of the newer agents of STD account for as many as or more infections seen in STD clinics or by private physicians than are accounted for by the classic causes. These infections are most common in the 15- to 30-year age group—the time of greatest sexual activity, especially extramarital. They are more commonly diagnosed in men, both because men tend to have more sexual partners than do women (except prostitutes) and because the lesions are more apparent in men. Multiple infections are common in both sexes, and gonorrhea and syphilis should be excluded by appropriate examination in every patient seen with an STD. The changing nature of and the increase in these infections have several causes, including the use of measures other than the condom for contraception, changing practices in heterosexual and homosexual activities, especially involving genital–oral and genital–anal contact, the importation of infection from Southeast Asia, and increased public confidence in the availability and effectiveness of antibiotic therapy.

The increasing importance of *Chlamydia* infections deserves further emphasis. Chlamydial genital infections include urethritis, acute epididymitis in men, and pelvic inflammatory disease in women; these are discussed in Chapter 10. Overall, about half the cases of urethritis in men are nongonococcal, and among male college students, this rises to 80–90%. *C. trachomatis* (immunotypes D–K) is responsible for 30–50% of symptomatic cases of nongonococcal urethritis (NGU) and is more common in white than in black men and in higher than in lower socioeconomic levels. Its incubation period is longer than that of gonorrhea, and symptoms of urethritis in males may appear 1–2 weeks or longer after penicillin or spectinomycin treatment for gonorrhea, involving one third to two thirds of these men. Oral therapy of NGU with tetracycline or erythromycin hastens recovery. There is no clinical counterpart of NGU in women who develop *Chlamydia* infections of the cervix with or without cervicitis, but this organism may be involved in up to 30% of pelvic inflammatory diseases in females.[89] The other agents of NGU in men are uncertain, although *U. urealyticum* is considered the cause of 10–20% of NGU cases. If laboratory facilities are not available for the diagnosis of *Chlamydia* infections in persons with NGU, both the case and the sexual partner should be treated with appropriate broad-spectrum antibiotic therapy (usually tetracycline or doxycycline) as though they had it. For detailed information on STD infections, see Chapter 9 on chancroid, Chapter 10 on *Chlamydia* infections, Chapter 15 on gonococcal infections, and Chapter 35 on syphilis. Viral causes are discussed in the companion book on viruses.[5]

11.2.7. Hospital (Nosocomial) Infections. These are infections acquired after an admission to a hospital

and are discussed in detail in Chapter 25. More than 2 million patients annually in the United States develop nosocomial infections. In the largest study to date, 5.7% of 159,526 patients in 338 hospitals developed a nosocomial infection.[90] Each such patient stayed an average of four extra days in the hospital, paying an extra $2,100 for each infection. Some 19,027 deaths were directly attributable to a nosocomial infection and it was a contributory factor in 58,092. In reports to the CDC's National Nosocomial Infections Surveillance program in 1991–1992, which involves 149 hospitals, urinary tract infections were the most common site (33.1%), followed by pneumonias (15.5%), surgical site infections (14.9%), and primary bloodstream infections (13.1%), and this order was similar irrespective of the size of the hospital. Among 71,411 isolates from all sites, *E. coli* and *S. aureus* were the most common (12% each), followed by coagulase-negative staphylococcus (11%), *Enterococcus* spp. (10%), *Pseudomonas aeruginosa* (9%), *Enterobacter* spp. (6%), and all other organisms 5% or less.[90] Unfortunately, the major nosocomial pathogens either are naturally resistant to clinically useful antibiotics or are potentially able to develop resistance. This includes both gram-positive organisms (*S. aureus, Enterococcus* spp.) and gram-negative organisms (*K. pneumoniae, P. aeruginosa*, and *Enterobacter* spp.).

12. Diagnosis of Bacterial Infections

Identification of the causal agent is essential to establish the etiology of a bacterial infection and as a guide to selecting appropriate antibiotic therapy. It depends primarily on (1) microscopic examination of exudates, body fluid, or tissues after staining (Gram's stain, acid-fast), or by dark-field examination or immunofluorescent-labeled antibody tests, or by the newer techniques for antigen identification such as counterimmunoelectrophoresis and latex agglutination, as used in respiratory infections; (2) appropriate bacteriological culture techniques; (3) a variety of new techniques to identify the antigen and a particular antigenic strain, many based on molecular methods; and (4) serological tests. Serological tests are not as commonly employed as in viral infections because of the ease and rapidity with which the diagnosis can often be established by smear and culture for most bacterial infections. For slow-growing or difficult-to-culture organisms such as *L. pneumophila*, the spirochetes, and the rickettsiae, serological tests play an important role in diagnosis. Animal inoculation may be required to identify fastidious organisms and the toxins of certain bacteria, and skin tests may be useful to diagnose infections that show delayed hypersensitivity.

This section will briefly review some of these techniques, but for more definitive information, see textbooks on laboratory methods,[91,92] medical microbiology,[32] or clinical infectious diseases.[38] An informative pamphlet on the services available from the CDC for the diagnosis of diseases of public health importance is available that indicates specimen collection, tests done, shipment instructions, and federal regulations on interstate shipment of etiologic agents.[93] All such specimens with proper justification and a completed request form must be submitted to the National Center for Infectious Diseases of the CDC in Atlanta by or through the state health laboratory with the knowledge and consent of the state laboratory director or designee.

An important decision for the physician is the differentiation of bacterial from viral infections on first examination in order to decide on the necessity of antibiotic therapy and as a guide to laboratory tests. This may not always be possible, but the presence of bacterial infections is suggested by the vigor and acuteness of the clinical onset, leukocytosis, and the presence of purulent lesions with polymorphonuclear cells. Nonpurulent responses to bacteria are seen in such diseases as brucellosis, tuberculosis, and typhoid fever. The presence of more than 10 white blood cells/mm^3 of urine by either hemocytometry or direct microscopy is indicative of an acute bacterial urinary infection,[86] as is the presence of white blood cells in the stool of a bacterial pathogen involved in diarrhea, or of polymorphonuclear leukocytes (not lymphocytes) in spinal fluid of a bacterial meningitis.

12.1. Collection of Specimens

The selection of the appropriate site from which to obtain the specimen, its collection prior to antibiotic therapy, and its transport to the laboratory in a manner to preserve the viability of any organisms present are three essential ingredients of successful diagnostic microbiology. The specimen should be taken from the site of the infection or from the body fluid most likely to contain organisms from the infected site. The collection should be made with sterile swabs and collecting units. Cotton applicator swabs are commonly used, but since cotton may be toxic to certain bacteria, a synthetic material such as calcium alginate is preferable. An adequate sample must be obtained to prepare smears and cultures and for special isolation purposes (viruses, fungi). Swabs may be inadequate for this purpose, and the fluid itself, or a washing of it (e.g., throat, nasal, lesion), may be needed for quantitative measurement, as in urine, to determine the number of organisms present. A syringe aspirate is desirable for anaerobic cultures of purulent lesions. Since most large laboratories have special sections for bacterial, viral, fungal, parasitic, and treponemal diagnostic

techniques, separate specimens for each microbiological group considered as a possible etiologic agent in a given infection should be collected. Special techniques may be needed to avoid contamination by normal flora in needle or surgical aspirations from a lesion or from a transtracheal or transurethral site.

To preserve viability, material from patients may be (1) inoculated into appropriate transport media directly at the bedside, for which purpose kits are now commercially available; (2) carried to the laboratory for immediate inoculation; or (3) preserved in a transport medium that maintains viability, prevents desiccation, and limits overgrowth of other organisms; media containing agar and charcoal are commonly employed (Stuart's transport medium or Arnie's modification thereof). If transport media are used, smears should be prepared separately at the time of collection with a separate swab because the agar in the transport medium makes this difficult. If viruses are suspected, a separate specimen should be collected and immediately frozen in dry ice for transport to the laboratory. Blood samples should be collected aseptically in amounts of 10–15 ml. If shipment to a laboratory is needed, the serum should be separated aseptically and forwarded preferably in the frozen state, but this will vary, depending on the infection. Infections of certain sites or with certain organisms may require special collection or transport methods or both; these are described in the appropriate individual chapters in this book or in books on bacteriological diagnosis.

12.2. Requests for Testing

There must be good communication between the physician or epidemiologist and the microbiologist. In the face of an outbreak, the epidemiologist is urged to consult with the laboratory prior to the investigation or have a laboratory specialist accompany the investigator to make the proper specimen collection and method of transporting it to the laboratory. The wide array of specialized laboratory techniques and culture media available makes it necessary that the laboratory be provided with information on a clinical and epidemiological assessment of the etiologic possibilities. The age, sex, clinical diagnosis (or at least the organ system involved), previous antibiotic therapy, and other pertinent patient information plus the site, time, and date of collection and method of transport to the laboratory of the specimen are helpful data to the laboratory worker in pursuit of the correct techniques to be employed. In return, the clinical microbiologist must provide periodic reports to help the physician in selecting appropriate therapy until the organism is fully identified and its antibiotic sensitivity determined.

12.3. Tests Employed

The details of laboratory methods are beyond the scope of this section, but a few comments will be made; specific techniques are mentioned in each chapter. The importance of a properly obtained and thoroughly examined Gram's stain of the exudate or body fluid cannot be overemphasized as a guide to initial therapy. For example, the etiology of some 85% of acute purulent meningitis cases and of many bacterial pneumonias can be identified by smear, especially if capsular swelling occurs in the presence of specific antisera. Simple microscopic examination of an unstained, uncentrifuged specimen of urine that shows bacteria indicates a quantitative bacterial count of 10^4–10^5/ml, and the morphology may point to the proper etiology. The examination of bacteria in fresh preparations or on culture, after staining with specific, immunofluorescent-labeled antisera, provides specific diagnosis of group A hemolytic streptococci, plague bacillus, and *E. coli*; it has also been useful in identifying the organism in acute meningitis, in cervical gonorrhea, and in primary syphilis.

The rapid detection of antigen in body fluids such as respiratory secretions and in urine is now being accomplished by immunofluorescence, counterimmunoelectrophoresis, enzyme immunoassays, DNA probe hybridization, and latex agglutination. Commercial kits are becoming rapidly available for many of these techniques.[94] A number of molecular techniques are now being used for both phenotypic and genotypic identification of organisms and are well reviewed in a recent article.[95] Those used for epidemiological studies of *phenotypic variants* include antibiotic resistance patterns (antibiograms), biotyping, bacteriocin production, serotyping, outer membrane protein analysis, phage typing, immunoblotting, and multilocus enzyme electrophoresis. The limitations of these techniques are that they do not take genetic exchange or mutations into account, are nonspecific, and are not widely applicable. Independent isolates of the same strain may vary in their phenotype. *Genotypic typing* methods are used to identify the genetic composition of the organism by study of chromosomal, plasmid, or transposon DNA. The techniques include plasmid profile analysis, restriction endonuclease analysis, ribotyping, pulsed field electrophoresis, polymerase chain reaction digests, arbitrarily primed polymerase chain reactions, and nucleotide sequence analysis. Variations in the reproducibility and discriminatory power of the two general methods differ; in general, those that involve genotypic methods are better. Their application permits identification of specific patterns of transmission of an organism, differentiation between reinfection and reactivation, and recognition of the geographic distribution of a particular

strain. The epidemiology of antibiotic resistance involves (1) R plasmid spread (inter- and intraspecies), (2) strain (clonal) dissemination, and (3) transposition of R genes into R plasmids or chromosomes. These many techniques now provide the epidemiologist the means for the rapid identification of many organisms, including the specific strain involved, so that transmission patterns can be followed. Of special interest are the so-called emerging infections, which in the United States include *E. coli* O157:H7 disease, cryptosporidiosis, coccidioidomycosis, multidrug-resistant pneumococcal disease, vancomycin-resistant enterococcal infections, and Hantavirus infections. Special surveillance and diagnostic centers have been established to monitor these infections.[96]

The culture media used will depend on the organisms suspected and whether they are aerobic or anaerobic. Blood agar plates and broth cultures are widely used as a starting point. See individual chapters for specialized media for specific organisms. A useful table indicating the bacteria found by common culture media (and the media used), by type of specimen (urine, respiratory secretions, genital, etc.), and the specialized media needed to detect organisms missed by these standard procedures is available in the appendix of a recent microbiology text.[32]

Once the organism is isolated its antibiotic sensitivity is usually determined and is useful not only in the selection of the proper antibiotic but also in "fingerprinting" the organism for epidemiological tracing. However, this is not necessary for some organisms that are known to be uniformly sensitive to certain antibiotics or that are uniformly resistant to all but one or two antibiotics. In vitro antibiotic testing is not always relevant to a particular infection, especially if the infection is due to mixed organisms. It often does not provide quantitative data or express the effect of an antibiotic on different points in the growth cycle of the organism, and the testing procedure may not include the antibiotic best suited to the clinical situation. The tests must be made on organisms obtained before antibiotic therapy is initiated. Despite these limitations, in vitro antibiotic testing is widely employed and is especially useful for organisms prone to develop antibiotic resistance such as staphylococci, enterobacteria, and *M. tuberculosis*.

The in vitro tests commonly employed are the disk-diffusion techniques in agar and dilution-sensitivity tests on agar plates or in tubes with nutrient broth. The disk procedure is simpler and more rapid, but the zone of inhibition cannot be directly correlated with the concentration of the antibiotic needed to inhibit growth of the organism in vivo. Antibiotic resistance to two antibiotics by disk diffusion does not exclude in vivo effectiveness of the combination; disk diffusion cannot be used for combinations of antibiotics.

The dilution tests provide quantitative data, permitting estimation of the minimum inhibitory concentration of the antibiotic as well as the minimum lethal concentration necessary for killing, which may differ from the minimum inhibitory concentration. The techniques used for dilution tests, however, may vary from laboratory to laboratory; the results are dependent on the size of the inoculum and the broth medium employed. Many laboratories now use automated broth microdilution susceptibility testing, which is rapid and has some economical and procedural advantages over both disk-diffusion and broth macrodilution methods.

As indicated earlier, serological tests have limited application for many bacterial infections. However, they are useful for a number of treponemal, leptospiral, and rickettsial infections. Some of the key tests are indicated in Table 11. Chlamydial infections, including the TWAR strain, are being increasingly recognized as a cause of a variety of clinical syndromes, especially of the respiratory and genital tracts, and microimmunofluorescence tests are available for both IgG and IgM antibodies as well as a genus-specific complement fixation test. Certain levels of titers of antibody are needed to be indicative of recent infection and differentiation between *C. trachomatis* and *C. psittaci* is not possible.

As with viral diagnostic tests, acute and convalescent serum samples should be collected for all bacterial serological tests and the sera tested simultaneously; a fourfold or greater rise in titer is indicative of recent infection. Remember that there is only one time to take the acute-phase sample—in early illness.

For legionellosis, the IgG immunofluorescence titer must reach 1:128 or higher to be significant because of nonspecificity at lower dilutions; similarly, only cold agglutinin titers of 1:64 or higher are regarded as diagnostic of *M. pneumoniae* infection, and even then only about 60% of hospitalized *M. pneumonia* patients are positive, depending on the severity of the infection. The presence of specific IgM antibody is often diagnostic of an acute infection, if demonstrable, as for *B. burgdorferi*, the cause of Lyme disease, but the laboratory diagnosis of this infection is subject to wide variation and standardization of procedures is badly needed. For rickettsial infections, both the organism itself and *Proteus* antigens are useful in serological diagnosis. In streptococcal infections, an increase occurs in a group of antienzyme tests but is too late to be useful diagnostically; a high titer is said to place the person at greater risk of developing rheumatic fever. In syphilis, a great variety of serological tests have been used, employing both nontreponemal and treponemal antigens. The VDRL test is most widely employed as an initial test and is highly sensitive and well standardized but lacks high specificity. The diagnosis can be confirmed by the fluorescent-treponemal-antibody absorption (FTA-Abs)

Table 11. Some Serological Tests Used in Bacteriological Diagnosis

Disease	Test antigen	Test(s)[a]	Comment
Brucellosis	Organism	Agglutination	Four strains
Legionellosis	Organism	IF	Twenty serotypes
Leptospirosis	Organism	Microagglutination, CF, hemolytic, IFA	Crossings occur; many types
Lyme disease	Organism	ELISA IgG, IgM	Needs standardization
Mycoplasma pneumonia	Organism	IF, TRI, CF	
	O rbc	Cold agglutinins	60% positive
Q fever	Organism	CF, agglutination	
Rickettsialpox	Organism	CF	
Rocky Mountain spotted fever	Soluble antigen	CF	
	Proteus strains	Weil Felix	Ox19+++, Ox2+++
		Agglutination	OxKO
Streptococcosis	Extracellular products	Antistreptolysin O, DNase	Confirm past infection
Syphilis	Nontreponemal	VDRL flocculation test	Presumptive
	Treponemal	Rapid reagent tests	Presumptive
		TPI, CF, FTA-ABS	Specific
Yaws	Same as syphilis	Same as syphilis	Cannot differentiate from syphilis
Tularemia	Organism	Agglutination	Often 1:640 or more
Typhoid fever	O antigen	Widal agglutination	≤Fourfold increase

[a]IF, immunofluorescence; CF, complement fixation; IFA, indirect fluorescent antibody; TRI, tetrazolium-reduction inhibition; VDRL, Veneral Disease Research Laboratory; TPI, treponemal immobilization; FTA-ABS, fluorescent-treponemal-antibody absorption.

or hemagglutination (MHA-TP) tests, which are highly specific and available at most state and a few large private laboratories, as well as at the CDC. The *Treponema pallida* immobilization test (TPI) test is a highly specific test but requires maintenance of a mobile, live treponemal antigen; it is available at the CDC. Yaws (*T. pertenue*) shares identical reactivities with *T. pallidum* and cannot be differentiated serologically. Typhoid fever results in increases to the H, O, and other antigens of the organism; an increase in O-antigen titer in the absence of recent immunization is indicative of recent infection. Skin tests may also be useful in diagnosis by demonstration of hypersensitivity to various bacterial antigens. They are commonly employed in tuberculosis, leprosy, nontuberculous mycobacteriosis, brucellosis, and tularemia.

12.4. Interpretation of Tests

The isolation of a bacterial organism from an ill person does not always represent a causal relationship. The organism could reflect (1) part of the normal flora; (2) a healthy carrier state; (3) contamination during the collection process; (4) a transient microorganism contaminating a body surface; (5) a dual or multiple infection, in which the organism isolated is not the one causing the clinical illness; (6) a laboratory error or mix-up; or (7) the true cause of the illness. The factors that point toward a causal relationship are (1) isolation in pure culture or of only one organism; (2) the presence of large numbers of the same organism; (3) the presence of the organism in direct smear from a lesion; (4) procurement from a site normally free of bacteria; (5) repeated isolation of the same organism; (6) demonstration of an immune response; and (7) history of possible recent exposure to the organism in an ill person, in travel, in occupation, or otherwise. The response of the patient to an antibiotic to which the organism is sensitive provides suggestive evidence that the organism caused the disease; but because other organisms are also sensitive to the same antibiotic, this cannot weigh too heavily. Clinical and laboratory judgment plus knowledge of the qualitative and quantitative behavior of human pathogens constitute the best grounds for deciding a causal relationship. It should be remembered that some organisms not usually regarded as pathogenic may cause disease in patients with naturally occurring or drug-induced defects in their immune defenses, or in patients infected with HIV.

On the other hand, the failure to isolate an organism does not exclude a bacterial etiology. Such "false-negatives" could result from (1) prior antibiotic therapy; (2) failure to obtain a specimen from the proper site; (3) collection at the wrong time; (4) a loss of viability during transport to the laboratory; (5) use of inappropriate media, temperature, gaseous

environment, or other conditions for growth of the organism; or (6) failure to hold the culture media for a sufficiently long time, as with certain *Brucella* species. For organisms difficult or impossible to culture, molecular tools such as the polymerase chain reaction are providing important techniques for direct diagnosis, but extremely careful laboratory procedures are needed to avoid cross-contamination and false-positive results.[6]

Serological tests are also subject to misinterpretation. False-positive rises can result from cross-reacting antigens, nonspecific inhibitors, double infection with the other organism causing the illness, and antibody response to vaccination rather than to natural infection. False-negatives, i.e., failure to demonstrate an increase in titer, can occur when the serum specimen is taken too late in illness, two samples are taken too close together to demonstrate a titer rise, the organism is a poor immunogen, the wrong antigen is used in the test, some inhibitor or nonspecificity (as in immunofluorescence tests) obscures a true rise, or the wrong type of test is used for the timing of the serum specimens. Serological tests are also not available for many bacterial infections. One of the common problems is the interpretation of a high IgG antibody titer in a single specimen. The demonstration of IgM-specific antibody is strong but not absolute evidence of a recent infection; it could result from reactivation. The presence of antibody to a rare infection, or to one not usually present in that area as in a returned traveler, or the history of some recent unusual exposure such as in hunting, a new occupation, or a visit from overseas friends also adds weight to the recency of that infection. Such problems are more common in viral and parasitic infections.

13. Proof of Causation

The classic postulates were suggested by Jakob Henle in 1840,[97] some 40 years before bacteria were discovered, and then further developed by his pupil, Robert Koch, in 1884 and 1890, after he had isolated *M. tuberculosis*,[98,99] as well as by Edwin Klebs.[100] A recent book has reviewed the development and subsequent changes in these postulates.[101] The postulates are presented in Table 12. While fulfillment of these postulates provides strong evidence of a causal association, the failure to fulfill them does not exclude this relationship. Even at the time of presentation in 1890, Koch himself recognized some of these limitations, especially the inability to reproduce some diseases in experimental animals. At that time, this was true of cholera, typhoid fever, diphtheria, leprosy, and relapsing fever, for which Koch felt it necessary to fulfill only the first two postulates. Since then, many other limitations have been recognized,[102] as our knowledge of microbiology, epidemiology, and pathogenesis has increased. These include recognition of the asymptomatic carrier state, which would invalidate the second postulate if an individual were carrier of organism A and his disease was caused by organism B. The concepts of multiple causation, of inapparent infection, and of the biological gradient of disease are also limitations. The postulates are not generally applicable to viral and parasitic diseases or for organisms that cannot be grown in culture or induce an immune response. Criteria are also needed for chronic diseases. In an effort to cover these various possibilities, a "unified" set of postulates based mainly on epidemiological criteria have been proposed.[103] It is clear that all postulates and guidelines of causation are limited by the technology available to prove them and by our knowledge of disease mechanisms at the time. The need for establishing guidelines for causation is ever present in the field of bacteriology, where the causal relationship between parasite and disease must be established for newly recognized diseases such as Lyme disease, Legionnaires' disease, Pittsburgh pneumonia, chlamydial pneumonia and urethritis, infant botulism, enterotoxic *E. coli* diarrhea, *H. pylori* and gastric ulcer and carcinoma, toxic shock syndrome, and streptococcal B infections in infancy. New causes for old diseases, new diseases from old causes, and new diseases with new causes keep appearing as our techniques for their isolation and identification improve.

Table 12. Henle–Koch Postulates[a]

1. Parasite occurs in every case of the disease in question and under circumstances that can account for the pathological changes and clinical cause of the disease
2. Occurs in no other disease as fortuitous and nonpathogenic parasite
3. After being fully isolated from the body and repeatedly grown in pure culture can induce the disease anew

[a]From Koch.[98,99]

14. Control and Prevention

The three major principles of control of infectious diseases are as follows: (1) eliminate or contain the sources of infection; (2) interrupt the chain of transmission; and (3) protect the host against infection or disease or both. Additionally, the environment may contribute to the occurrence of disease by its effect on any of the above three areas.

14.1. Environmental Control

The provision of clean and safe air, water, milk, and food; the proper management of sewage and garbage; and the

control of insect vectors of disease are regarded as not only essential to health but also a legal right in most countries. The extent to which they are attained depends on the economy, energy resources, political will, and educational level of the country.

14.1.1. Air. While many infectious agents are airborne on droplet nuclei or particles from infected hosts or environmental sources to susceptible subjects, effective control of this means of transmission has been most difficult to achieve. In open environments, it has been impossible to attain, and even in closed environments attempts to sterilize the air by UV light filtration, propylene glycol, and other chemical aerosols have met with very limited success. At best, control of the air currents generated by air conditioners, water coolers, and fans will help slow down spread of organisms such as *L. pneumophila, M. tuberculosis*, and staphylococci. For patient isolation, laminar-flow units have been effective if properly used. The pollution of air by automobiles and industrial sources does not carry a direct risk of infection, but may depress host defense mechanisms so that disease develops, as in persons with chronic pulmonary diseases.

14.1.2. Water. Improvement in our water supplies has been one of the major factors in the environmental control of infectious diseases, especially of enteric infections such as *Campylobacter*, yersiniosis, cholera, typhoid fever, amebiasis, bacillary dysentery, as well as waterborne viruses like Norwalk viruses. The details of water purification and treatment can be found elsewhere, but involve removal of extraneous materials by filtration, settling, and coagulation, the replenishment of oxygen by aeration, and disinfection by chlorination. Bacteriological and chemical standards for the purity of water have been established and are reinforced by governmental and legal regulations. The presence of *E. coli* in defined numbers per milliliter[2] is taken as an index of fecal contamination of water. However, its absence does not guarantee water safety, since organisms such as hepatitis A and E viruses may escape filtration and chlorination procedures, and more recently *Cryptosporidium* cysts escaped a filtration system in a public water supply in Milwaukee, causing a massive outbreak of diarrhea involving over 403,000 persons.[28] This organism is also resistant to tolerable levels of chlorine. For the traveler, treatment of water with chlorine-release tablets or a drop of Lugal's iodine solution per quart of water 30 min prior to use or by boiling for 5 min (portable heating units can be purchased) will decrease the likelihood of acquiring bacterial intestinal infections in developing countries. Small millipore filter kits are also available for the traveler and are said to remove bacteria, *G. lamblia*, and even some viruses. Should these methods not be available, one can allow the water to run until very hot, collect it, and then use it for drinking water. Bottled water, wine, and beer or other bottled or canned beverages are usually safe.

14.1.3. Sewage and Garbage. Sewage water carries fecal material, industrial and chemical products, and other waste products. It must be safely conveyed without human hazard to septic tanks or to reprocessing, filtration, and activated sludge treatment centers, but has not posed a problem of infectious disease unless cross-contamination with water pipes occurs. The dumping of untreated sewage into streams, rivers, and the open sea is detrimental to fish and marine life and esthetically distasteful and impairs recreational use of such waters. It might result in transmission of certain intestinal pathogens via raw seafood to humans, such as the hepatitis A or E viruses, cholera, or *Cryptosporidium* in water itself. Clams and oysters have been responsible for many outbreaks of this sort, and thorough cooking is needed to kill organisms such as cholera or hepatitis viruses.

The proper disposal of the large amount of garbage, refuse, and other solid waste products produced by our modern society is an increasing challenge to proper land use and modern technology. Direct discharge into the sewage system after grinding and flushing is one method; compactors of garbage and waste material are now available even for home use. If stored and carried elsewhere for disposal, this must be done in closed, stable containers that protect against rats, flies, and other predators. The separation and recycling of certain reusable products such as glass, bottles, paper, and cans is to be encouraged. Large incinerators for burning garbage and refuse provide a safe but energy-expensive procedure. Landfill sites in communities are increasingly scarce, costly as land values increase, and objected to on esthetic grounds. Disposal of radioactive and other toxic wastes generated by medical, industrial, and energy uses in oceans raises international concern about the long-term food and energy potential of oceans, and land or tank disposal, especially of nuclear wastes, is unacceptable to most communities. The increasing uses of pesticides, and domestic and wild animal wastes in streams or rivers that may wash down into public water supplies, pose an increasing threat.

14.1.4. Milk and Food. Milk and food must be protected against contamination at their source, during transport and storage, and in their preparation for consumption. Cows must be free from tuberculosis, brucellosis, and Q fever. The milk must be collected under clean conditions, preferably by

[2]Usually, water is not acceptable for drinking if coliform bacteria exceed 4/100 ml in more than 5% of water samples per month using the membrane-filter technique; samples should be taken at points representative of the distribution system.[104]

automated machines that avoid human contamination, and its quality in the raw state and after pasteurization must meet bacteriological standards.[3] Common pasteurization procedures are heating to 65°C (149°F) for 30 min or the high-temperature or flash method at 72°C (162°F) for 15s. The heat inactivation of the enzyme alkaline phosphatase, normally present in milk, provides the basis for the phosphatase test to ensure proper pasteurization. Before and promptly after pasteurization, milk must be stored and transported at 5–10°C (41–50°F) to storage areas or the consumer. The pasteurization process, even if performed correctly, may not decontaminate some intracellular organisms and those that are cold tolerant, such as *Listeria*. Milk and other food products may contain antibiotics used in treatment of cattle or for growth promotion and pose a hazard to highly sensitive persons.

Our food supplies must also be properly grown, cultivated, stored, and prepared. In developing and tropical areas, vegetables such as lettuce and other products grown in soil enriched by human excreta pose a serious hazard for intestinal infections, and these foods should be avoided by wary travelers. Our modern supermarkets are now selling foodstuffs of all types that have been rapidly imported from other countries and that may carry pathogens. In the summer months, 75% of many fruits and vegetables are harvested outside the country and delivered within days to grocery stores and restaurants in the United States.[105] Salad bars, now present in most restaurants, provide fruits and vegetables that may have originated in Mexico, Central America, or tropical or semitropical climes and are a major source of food poisoning.[106] The fast-food chain, particularly those serving undercooked hamburgers, have been the source of food outbreaks, particularly due to *E. coli* O157:H7 infection in the United States[107]; this organism has also led to an epidemic in a child care center. Foodborne outbreaks have also been due to shigellosis on a commercial airline,[108] and to *Salmonella* in a multistate outbreak due in contaminated cheese.[109] These developments have led to a changing epidemiology of foodborne disease and to new approaches to prevention.[110,111] Human handling during preparation may result in contamination by many bacteria, especially staphylococci, *Salmonella*, and *Shigella*. Thorough cooking immediately prior to eating will reduce the hazard of infection but may fail to inactivate preformed heat-stable toxins such as *C. perfringens*. Proper refrigeration or freezing of food has been as important as water and sewage management in the reduction of intestinal diseases. It prevents the multiplication of most bacteria, and freezing often destroys some parasites such as *Toxoplasma gondii* and *Trichinella spiralis*. However, most organisms or toxins already present at the time of refrigeration or freezing will be preserved in the process and can multiply after thawing and reaching the proper temperature.

[3]Under 200,000 bacteria/ml by standard plate count before pasteurization and under 30,000 bacteria/ml after pasteurization.

14.1.5. Animals and Insect Vectors. Animals provide the source of infection for many of the diseases discussed in this book, such as anthrax, brucellosis, *Campylobacter* infection, leptospirosis, plague, salmonellosis, tularemia, and yersiniosis. The types of exposure differ: some are occupational (anthrax) or avocational (tularemia), some result from common environmental exposures (leptospirosis, salmonellosis), some from close contact with domestic animals (*Campylobacter*), or some from contamination of water sources (*Campylobacter*, yersiniosis) by animals. Knowledge of these potential sources of infection and of appropriate specific measures to avoid or minimize exposure is needed.

Insect vectors may play a passive or active role in transmission of bacterial infections. Passive transfer of the organisms of cholera, salmonellosis, and typhoid fever by flies and other insects may occur but does not seem to be of much epidemiological significance. Good food sanitation, proper storage, and screens are useful to prevent such transfer. The active transport of infection involves multiplication in the insect host. Rickettsial infections are commonly transmitted by insect vectors: *R. rickettsii* of Rocky Mountain spotted fever by wood, dog, and lone star ticks; *R. akari* of rickettsialpox by the mouse mite; and *R. prowazekii* of typhus fevers by the body louse (louse-borne typhus) or rat flea (murine typhus). *B. burgdorferi*, the spirochete that causes Lyme disease, is transmitted by ticks of the *Ixodid* genus, such as *I. dammini*, the deer tick in eastern and midwestern states, and by *I. pacifus* in western states. Ticks may also transmit ehrlichiosis (Sennetsu fever) and babesiosis, and cases have been recognized in the eastern United States.

The control of many of these insect vectors is very difficult, since they exist widely in nature and in many domestic gardens and lawns in endemic areas. Careful protection of the body, especially wearing long pants and high stockings, along with an antitick insecticide in summer months in outside work is important in prevention of tick bites. Intensive searching of the body, as well as domestic pets, after potential exposure is important in preventing Lyme infection, since the tick must remain on the skin some 24 h before it can transmit the organism. Attention should also be directed at the control or avoidance of the dog, rat, mouse, deer, or other animal hosts on which the ticks usually reside. The body louse is controlled by good personal and clothing hygiene and by delousing procedures (heat and chemical treatment).

14.2. Host Factors

The human host may be protected against infection and disease by quarantine or isolation from the sources of infection, by good personal hygiene (especially hand washing), by specific immunization, and by chemoprophylaxis.

14.2.1. Quarantine and Isolation. Quarantine, which began around 1,348 as a 40-day period of keeping suspected plague victims aboard ships or in house quarantine in Venice, has now been rendered largely obsolete by air travel. The WHO now evokes quarantine measures only for plague, yellow fever, and cholera. We rely on surveillance techniques, as discussed in Chapter 2 and by Teutsch,[16] to provide constant monitoring and analysis of infectious diseases and of other conditions of public health importance. The emergence of new and antibiotic-resistant infectious agents[10] has alerted us to the deficiencies in our current surveillance system in the United States,[112] as well as in Europe.[15] A prevention and surveillance strategy has been developed by the CDC for these emerging infections.[96]

Isolation requirement and techniques have also undergone redefinition and reassessment (see Chapter 25 and recent books for details).[113,114] Varying isolation standards in hospitals have been developed for various groups of diseases and often depend on state laws or hospital regulations. Infectious diseases in hospitals have been grouped according to the degree of isolation recommended. Standard precautions are recommended for all patients regardless of their diagnosis. Specific precautions, "transmission-based precautions," are recommended for specific situations. Hand washing remains as a recommendation following any patient contact.

Standard precautions are now legally mandated based on the assumption that the blood and certain body fluids (amniotic fluid, pericardial fluid, peritoneal fluid, pleural fluid, cerebrospinal fluid, semen, vaginal secretions, and any body fluid visibly contaminated with blood) are potentially infected, irrespective of the patient from whom it is derived. With this in mind, the CDC has issued recommendations for the prevention of transmission of HIV, hepatitis B, and other blood-borne pathogens in medical care settings.[115] These standard precautions are to be applied to all patients, including those coming into emergency rooms, where there is often risk of such exposures and the status of the patients is usually unknown. The approach also applies to outpatient settings that involve handling of blood or body fluids. Standard precautions specifically involve the use of gloves whenever contact with blood or other such body fluids is anticipated and other barrier techniques (masks, gowns, and protective eyewear) whenever splashes of such fluids are expected. This would apply to the use of gloves when touching blood and body fluids, mucous membranes, or soiled articles or surfaces, and when doing venipunctures or inserting intravenous lines, and to the use of masks in operative or invasive procedures. The decision to initiate routine HIV testing or testing of high-risk patients and high-risk hospital personnel engaged in invasive or other procedures is left to physicians or individual institutions. If such testing is carried out, the CDC outlines the principles of informed consent and confidentiality involved.[116]

14.2.2. Hygiene. High standards of cleanliness of the individual, the family, the food prepared, and the community contribute to the prevention of infectious diseases. The simple measure of thorough and frequent hand washing is of the highest importance in protecting the individual against pathogens and in interrupting the spread of organisms to others. It plays an essential role in controlling the spread of infection in hospitals and institutions, in restaurants, and in food processing both in industries and at home. The promotion, distribution, and proper use of soap and water for personal hygiene in developing countries need greater emphasis. Its use should decrease enteric and respiratory infections.

14.2.3. Immunization. The specific protection of the individual against infection and disease is the key to modern preventive practice. It may be either passive protection by the transfer of a specific antibody (or antitoxin) from another person or animal immune to it or active immunization through induction of antibody by the organism itself or an antigenic derivative of it. An ideal vaccine closely simulates the protection from natural infection, i.e., it produces good humoral, cellular, and local immunity of long duration. Preferably, it should be better than the short-lived or incomplete immunity found in certain infections such as cholera or shigellosis. It should be in a form of administration and at a cost acceptable to the public. The cost of the vaccine and any side effects should be less than those of the natural disease prevented by it. These ideals are most closely met by well-attenuated live vaccines or antigenic derivatives thereof. The live bacterial vaccines include bacille Calmette–Guérin (BCG) for tuberculosis, a recently developed and licensed oral vaccine (using strain Ty21a) for typhoid fever, and tularemia vaccine. BCG is sparsely used in the United States, and controversy exists about its efficacy (see below). Tularemia vaccine is used in rather small, high-risk groups. Most bacterial vaccines are formalin-, acetone-, or phenolkilled organisms or an antigenic derivative such as the capsular polysaccharides of the meningococcus and pneumococcus (23 types), the toxoids of diphtheria and tetanus, or the protein antigens of *B. anthracis*.

Newer or improved vaccine preparations for many bacterial diseases are under development, testing, or licensure such as those for cholera, *H. influenzae* type b

conjugate (now licensed and used) pertussis, shigellosis, and typhoid fever. The use of conjugated polysaccharide vaccines improves their antigenicity, permitting them to be used in young children.

Someday, timed-release biodegradable polymers may permit pulsed release of vaccines, such as tetanus, thus permitting a single shot to be effective, and avoid the loss now occurring in women and children who do not return to complete the multiple doses required for most killed vaccines. The Institute of Medicine of the U.S. National Academy of Sciences has published a comprehensive evaluation of vaccine priorities for both developed and developing countries based on their feasibility, cost, need, effectiveness, acceptability, side reactions, and other factors.[117]

In developing countries, the WHO's Expanded Program in Immunization is making an enormous effort to vaccinate the young children of the world against six targeted diseases using diphtheria–pertussis–tetanus (DPT), polio, measles, and BCG vaccines, as well as hepatitis B and yellow fever vaccine in some regions. Important progress is being made, and the worldwide coverage in 1996 was approximately 70%. Other organizations are now joining in this effort, as part of a "Child Survival Program," and there is a focus on "growth, oral rehydration therapy, breast feeding, and immunization" (GOBI). The Children's Vaccine Initiative, the organization that manages the technological transition toward new vaccines, has outlined the desired characteristics of such vaccines and has stated that they should be capable of being given orally in the first year of life. In the United States, the recommendation by states that all children be immunized before being allowed into school has greatly expanded coverage in this country. Of the nine diseases against which children are routinely vaccinated, the reported number for five of these and for congenital rubella syndrome was at or near the lowest levels ever.[118] The reported number of these five diseases in 1996 were diphtheria (2 cases), measles (508 cases), poliomyelitis (wild virus—0 cases), rubella (238 cases), and tetanus (36 cases). Only seven cases of congenital rubella syndrome were reported. Measles is a good example of the progress made. After an outbreak of measles between 1989 and 1991 that involved more than 50,000 cases and 11,000 hospitalizations, reported cases declined from 9,643 cases in 1991 to only 281 in 1993.[118,119] Rapid progress is being made in the reduction of meningitis caused by type b *H. influenzae* due to the widespread use of Hib vaccines.

In a survey in 1987, however, serious vaccine deficiencies were found in adults.[120] For example, sero-surveys indicated that 49–66% of persons 60 years or over lacked reliable protective levels of circulating antitoxin against tetanus, and 41–84% lacked adequate protection against diphtheria. Tetanus is a completely preventable disease, and persons over 50 account for 70% of reported cases; thus, special emphasis must be placed on this age group for tetanus boosters, or an initial series if not previously vaccinated. Pneumococcal vaccine is also badly underutilized as indicated by the fact that less than 10% of the higher-risk groups have been vaccinated. A similar lack of protection of our adult population exists against many viral diseases, especially influenza, measles, mumps, rubella, and hepatitis B, as well as pneumonia. Today, some 50,000–70,000 adults die each year of influenza, pneumococcal infections, and hepatitis B, with a cost to society of these and other vaccine-preventable diseases of adults exceeding 10 billion dollars each year.[121] Detailed recommendations have been issued to improve this situation[121] and a call for action issued by the CDC.[122]

Recommendations for the immunization of persons with altered immunocompetence, such as AIDS, have been made by the Advisory Committee on Immunization Practice (ACIP). In general, live viral or bacterial vaccines are contraindicated in persons with severe immunodeficiency due to any cause, including HIV-infected persons. However, evaluation and testing for HIV infection of asymptomatic persons are not necessary before administering measles–mumps–rubella (MMR) vaccine, since no documented serious adverse reactions have been noted in either asymptomatic or symptomatic persons infected with HIV. Thus, MMR vaccine is recommended for both adults and children when otherwise indicated, regardless of HIV status. Enhanced, inactivated poliomyelitis vaccine is preferred in HIV-infected persons, as is pneumococcal vaccine of these persons over age 2 years. HIV-infected children under 2 years should receive Hib vaccine according to the routine schedules. Both HIV-infected children may have suboptimal immune responses to such vaccines. Recent studies suggest, however, that the benefits of measles, mumps, and rubella vaccines outweigh the risks to children with AIDS, at least in developing countries. It should be noted that in developing countries where the risks of exposure are higher, greater use of live vaccines is recommended. The WHO thus recommends use of standard Expanded Programme on Immunization (EPI) vaccines in persons with symptomatic or asymptomatic HIV infections,[123] but suggests that inactivated poliomyelitis vaccine (IPV) be considered as an alternative to oral polio vaccine (OPV). Some complications have arisen with BCG vaccine in such immunosuppressed children, and its use should be suspended in unimmunized individuals with symptomatic AIDS in countries where the other targeted diseases remain serious risks; in asymptomatic HIV-infected individuals in areas where the risk of tuberculosis is high, BCG is recommended at birth or soon thereafter. Guidelines should be consulted for updated recommendations, as data are often

incomplete. Separate needles are required to avoid the possibility of parenteral transmission of HIV (and hepatitis B virus). The jet gun should not be used in immunization programs, except in an epidemic emergency, until further data are available on the possible risks associated with its use.

Table 13[124] gives the current recommended schedule for active vaccination of infants and children. Standards for pediatric immunization have also been issued by the same committee.[125] Information for international travelers,[126] as well as the uses and limitations of each vaccine, will be found in the appropriate chapters of this book. A brief summary follows.

14.2.3.1. Anthrax. No confirmed cases reported in the United States since 1988. The vaccine is distributed only by the Division of Biologics, Michigan Department of Public Health, and is intended only for high-risk occupational exposures, such as persons working with imported goat hair, wool, and hides (sheep and goats) and laboratory workers regularly exposed to this organism. The vaccine is a killed cell-free culture filtrate prepared from the protective antigen. A recent review of anthrax is available.[127] (See also Chapter 4.)

14.2.3.2. Botulism. There are three forms: classical, infant, and wound; a total of 119 cases were reported in 1996. Passive immunization is available consisting of horse serum with anti-A, B, and E toxins and used for persons strongly suspected of botulism or when disease is first diagnosed. Trivalent antitoxin is available from the CDC in Atlanta.

Table 13. Recommended Childhood Immunization Schedule, United States, 1997[a]

	Age										
Vaccine	Birth	1 mo	2 mos	4 mos	6 mos	12 mos	15 mos	18 mos	4–6 yrs	11–12 yrs	14–16 yrs
Hepatitis B[b,c]	Hep B-1		Hep B-2		Hep B-3					*Hep B*[c]	
Diphtheria, and tetanus toxoids and acellular pertussis[d]			DTap or DTP	DTap or DTP	DTap or DTP		DTap or DTP		DTap or DTP	Td	
Haemophilus influenzae type b[e]			Hib	Hib	Hib	Hib					
Poliovirus[f]			Polio[f]	Polio			Polio		Polio		
Measles, mumps, rubella[g]						MMR			MMR or MMR		
Varicella virus[h]							Var			***Var***	

[a]This schedule indicates the recommended age for routine administration of currently licensed childhood vaccines. Some combination vaccines are available and may be used whenever administration of all components of the vaccine is indicated. Providers should consult the manufactures' package inserts for detailed recommendations. Vaccines are listed under the routinely recommended ages. Bars indicate range of acceptable ages for vaccination. Shaded bars indicate catch-up vaccination: at 11–12 years, hepatitis B vaccine should be administered to children not previously vaccinated, and varicella virus vaccine should be administered to unvaccinated children who lack a reliable history of chickenpox. Use of trade names and commerical sources is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services. Source: Advisory Committee on Immunization Practices (ACIP), American Academy of Pediatrics (AAP), and American Academy of Family Physicians (AAFP).

[b]*Infants born to hepatitis B surface antigen (HBsAg)-negative mothers* should receive 2.5 μg of Merck vaccine (Recombivax HB®) or 10 μg of SmithKline Beecham (SB) vaccine (Energix-B®). The second dose should be administered >1 month after the first dose. *Infants born to HBsAg-positive mothers* should receive 0.5 ml hepatitis B immune globulin (HBIG) within 12 h of birth and either 5 μg of Merck vaccine (Recombivax HB®) or 10 μg of SB vaccine (Engerix-B®) at a separate site. The second dose is recommended at age 1–2 months and the third dose at age 6 months. *Infants born to mothers whose HBsAg status is unknown* should receive either 5 μg of Merck vaccine (Recombivax HB®) or 10 μg of SB vaccine (Engerix-B®) within 12 h of birth. The second dose of vaccine is recommended at age 1 month and the third dose at age 6 months. Blood should be drawn at the time of delivery to determine the mother's HBsAg status; if it is positive, the infant should receive HBIG as soon as possible (no later than age 1 week). The dosage and timing of subsequent vaccine doses should be based on the mother's HBsAg status.

[c]Children and adolescents who have not been vaccinated against hepatitis B during infancy may begin the series during any childhood visit. Those who have not previously received three doses of hepatitis B vaccine should initiate or complete the series at age 11–12 years. The second dose should be administered at least 1 month after the first dose, and the third dose should be administered at least 4 months after the first dose and at least 2 months after the second dose.

[d]Diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP) is the preferred vaccine for all doses in the vaccination series, including completion of the series in children who have received one or more doses of whole-cell diphtheria and tetanus toxoids and pertussis vaccine (DTP). Whole-cell DPT is an acceptable alternative to DTaP. The fourth dose of DTaP may be administered as early as 12 months of age, provided 6 months have elapsed since the third dose and if the child is considered unlikely to return at age 15–18 months. Tetanus and diphtheria toxoids (Td), absorbed, for adult use, are recommended at age 11–12 years if at least 5 years have elapsed since the last dose of DTP, DTaP, or diphtheria and tetanus toxoids. Subsequent routine Td boosters are recommended every 10 years.

[e]Three *H. influenzae* type b (Hib) conjugate vaccines are licensed for infant use. If PRP-OMP (PedvaxHIB® [Merck]) is administered at ages 2 and 4 months, a dose at age 6 months is not required. After completing the primary series, any Hib conjugate vaccine may be used as a booster.

[f]Two poliovirus vaccines are currently licensed in the United States: inactivated poliovirus vaccine (IPV) and oral poliovirus vaccine (OPV). The following schedules are all acceptable by ACIP, AAP, and AAFP, and parents and providers may choose among them: (1) IPV at ages 2 and 4 months and OPV at age 12–18 months and at age 4–6 years; (2) IPV at ages 2, 4, and 12–18 months and at age 4–6 years; and (3) OPV at ages 2, 4, and 6–18 months and at age 4–6 years. ACIP routinely recommends schedule 1. IPV is the only poliovirus vaccine recommended for immunocompromised persons and their household contacts.

[g]The second dose of measles–mumps–rubella vaccine is routinely recommended at age 4–6 years or at age 11–12 years but may be administered during any visit, provided at least 1 month has elapsed since receipt of the first dose and that both doses are administered at or after age 12 months.

[h]Susceptible children may receive varicella vaccine (Var) during any visit after the first birthday, and unvaccinated persons who lack a reliable history of chickenpox should be vaccinated at11–12 years. Susceptible persons aged≥13 years should receive two doses at least 1 month apart.

There is a 10–15% risk of adverse reactions (anaphylaxis, serum sickness). Botulism toxoid is also available and effective, but because of the rarity of the intoxication, it is recommended only for laboratory workers and others working directly with the toxin. It can be obtained from the CDC. (See also Chapter 6.)

14.2.3.3. Cholera. The available inactivated vaccine offers rather poor protection (about 50%) over a short period (3–6 months); the transmission of infection is not prevented. No country or territory currently requires immunization for entry.[126] However, local authorities may require it, and one dose satisfies such regulations and is valid for 6 months. For persons highly exposed in highly endemic areas, a three-dose primary series is given 1 week to 1 month apart and a booster dose every 6 months. The vaccine is probably not effective against the newly discovered *V. cholera* 039 strain. A number of oral cholera vaccines are under development and testing, some of which contain the *V. cholera* 039-Bengal strain.

14.2.3.4. Diphtheria–Pertussis–Tetanus (DPT) Vaccine (see Table 13). Routine primary immunization with DPT vaccine is given at 2, 4, and 6 months with a fourth dose at 15 months and a booster at 4–6 years before entry into school.[124] Some 20% of adults or more may be unprotected against diphtheria and need either a full primary series or a booster dose of a preparation for adults or children >7 years old (Td). Booster doses of Td are suggested for all adults every 10 years.[121] Local Arthus-type reactions and sometimes systemic immune complex reactions (serum sickness) may occur in hyperimmunized adults. These may be attended by severe head, muscle, and joint aches and fever of about 24 h duration. Corticosteroids may be given orally over 3–5 days, for symptomatic relief. Diphtheria antitoxin should be given to asymptomatic, unimmunized contacts in whom close surveillance is not possible, plus penicillin (600,000 U of the benzathine form or, if sensitive, a 7-day course of erythromycin) and injection of diphtheria toxoid. If close surveillance is possible, the antitoxin can be omitted.

14.2.3.5. Haemophilus influenzae Type b Vaccine (Hib). This infection is an important cause of meningitis in children, particularly those under the age of 5 years, and is now being recognized as important in older persons whose immunity has waned. Two forms of conjugated vaccines are available, and two in combination with DPT. They are given at 2, 4, and 6 months with DPT and OPV and again at 12–15 months when MMR is given. One preparation (PRP–OMP) is not given at 6 months (see Table 13 on routine active vaccination of infants and children). (See also Chapter 16.)

14.2.3.6. Meningococcal Infections. Meningococcal infections can result in epidemics but, in U.S. civilians, most commonly occur as single cases or localized clusters, with a third of the cases occurring in persons 20 years of age or over. Two polysaccharide vaccines are currently available in the United States: a bivalent A-C and a quadrivalent vaccine containing A, C, Y, and W-135 polysaccharides. A single dose of either is adequate to induce serospecific immunity.[32] Routine vaccination is not recommended in the United States because of the relatively low risk of infection and because a good group B antigen is not available. Vaccine usage is recommended as an adjunct to antibiotic chemoprophylaxis for household and other close contacts of persons with meningococcal disease due to serotypes A, C, Y, and W-135. The quadrivalent vaccine is recommended for travelers to endemic areas. The duration of protection is unclear. Side reactions are infrequent and mild, but the safety for pregnant women has not been established. Because of the high risk in military recruits, they have received meningococcal vaccine on entering the services since the early 1970s. Currently, they receive groups A, C, Y, and W-135. (See also Chapter 23.)

14.2.3.7. Plague. The vaccine consists of formalin-inactivated organisms; its efficacy has not been critically evaluated. It should be given only to high-risk groups such as field and laboratory personnel exposed to the organism and possibly to workers in plague enzootic or endemic rural areas where avoidance of rodents, fleas, and wild rabbits is not feasible (agricultural advisors, Peace Corps volunteers, or military personnel on maneuvers).[24] The schedule consists of five injections, with dosage varying with age. In the face of continued exposure, single booster doses at about 6-month intervals are given for two doses, then at 1- to 2-year intervals. Local reactions are common, sterile abscesses are rare, and systemic reactions (fever, headache, and malaise) may occur on repeated injections. (See also Chapter 27.)

14.2.3.8. Pneumococcal Infections. The estimated annual incidence of pneumococcal pneumonia in the United States is 68–260 cases per 100,000 population and of bacteremia is 7–25 per 100,000. Mortality is highest in patients with bacteremia, meningitis, underlying medical conditions, and those over 60 years of age. The currently available pneumococcal polysaccharide vaccine contains purified capsular materials from 23 types of *S. pneumoniae*, which together account for 87% of recent bacteremic pneumonia in the United States. The vaccine is particularly recommended for three groups: (1) adults with chronic diseases, especially of the cardiovascular or pulmonary systems; (2) adults with chronic illnesses specifically associated with an increased risk of pneumococcal infection or its complications (splenic dysfunction, Hodgkin's disease, multiple myeloma, cirrhosis, alcoholism, renal failure, CSF leaks, and in immunosuppressed patients); and (3) older adults, especially those 65 years of age and over, regardless of whether they are healthy or diseased. Vaccination is recommended

for hospitalized patients in these high-risk groups before discharge. A single dose is recommended without a booster. Mild side reactions consisting of erythema and pain at the site of injection occur in about half the recipients. Medicare helps pay the cost in these designated groups. Pneumococcal infections are also a problem in young children in developing countries, so that there is increasing interest in the use of the vaccine in these groups. (See also Chapter 28.)

14.2.3.9. Tetanus. See DPT (see Section 14.2.3d) for routine immunization. For wound management, tetanus–diphtheria (Td) adult-type, or tetanus toxoid (TT) only, is used alone or in combination with tetanus immune globulin (TIG) in doses of 250 U (in separate site and syringe), depending on the severity of the wound and the history of prior immunization. Moderate to severe local and systemic reactions may occur in some hyperimmune adults receiving booster doses or for wound prophylaxis. (See also Chapter 37.)

14.2.3.10. Tuberculosis. This disease remains the most important cause of death in the world due to an infectious agent. It is a major killer of individuals in persons aged 20–30 years. In 1994, the WHO declared this disease to be a public health emergency,[128] and it is believed to be "out of control" in many parts of the world. The WHO identified 10 industrial countries in which cases of tuberculosis increased 5–33% in recent years. In the United States, an estimated 15 million persons are believed to be infected. The increase of infection in HIV-infected persons with the emergence of multidrug-resistant strains in this group and in others who were inadequately treated is of special concern. Such resistant strains spread readily to others. About 4.5% of tuberculosis in the United States was associated with HIV infection, and a rise to 13.8% is anticipated by the year 2000. As Comstock[129] has pointed out, the patterns of change and reasons for it are multiple and complex both in the United States and other areas of the world. While HIV is widely blamed, there are many other factors such as migration, poverty, malnutrition, and decreased tuberculosis control efforts, and it is difficult to assess the relative importance of each in the trends observed. In 1991, 56% of active cases were reported among Hispanics and African Americans. The only vaccine available is BCG, and contradictory evidence exists as to its efficacy. An excellent brief review has recently summarized the evidence.[130] More people have received BCG vaccine than any other vaccine, yet its efficacy remains in doubt. It is a part of the routine WHO EPI program. For example, the WHO estimates that 85% of all children born in 1990 received BCG vaccine in their first year of life. Estimates of its efficacy range from 0 to 80% in different settings. Such great variation has been attributed to different vaccine strains, methodological differences, variation in the infecting organism in different countries, the genetic background of the exposed groups, and environmental interactions with other mycobacterial strains in the population. Indeed, a higher efficacy has been seen against leprosy than tuberculosis in some studies. One recent meta-analysis involving 14 trials and 12 observational studies concluded that BCG reduced the risk of tuberculosis by 50%,[131] despite great heterogeneity in the data. In another meta-analysis of results showing homogeneity, BCG was 86% effective against miliary tuberculosis and tuberculous meningitis; the preventive effect against pulmonary tuberculosis showed such heterogeneity that they did not calculate a summary measure.[132] It seems reasonable to believe that BCG is effective against the spread of tuberculosis within the body, such as miliary spread and spread to the meninges, but that its protection against infection varies greatly in different settings and with different vaccines. Comstock[129] has discussed the use of BCG and the CDC has outlined its use in control in a joint statement with the ACIP and the Advisory Committee on the Control of Tuberculosis.[133] General problems in the production standards for BCG and the need for recombinant technology in the development of a vaccine whose mechanism of action and efficacy can be better understood and evaluated have been recently presented.[134–136] BCG use is not routinely recommended in the United States because of the generally low incidence of disease and because BCG interferes with the interpretation of the tuberculin skin test. These recommendations may have to be modified for high-risk groups, in view of recent increases in incidence and resistant organisms. (See also Chapter 39.)

14.2.3.11. Typhoid. Not recommended for routine use in the United States or for international travel except for areas where there is a recognized risk of exposure to *Salmonella typhi*. It is recommended for travelers who will have prolonged exposure to potentially contaminated food and water in smaller cities or villages in rural areas in Africa, Asia, and Central and South America. An oral, live attenuated vaccine is licensed containing the Ty21a strain. It is taken in an enteric-coated capsule on alternate days for four doses, with a booster dose every 5 years if exposure continues.[137] An inactivated parenteral vaccine is also available and soon a Vi capsular polysaccharide vaccine will be available. Few reactions are due to the oral vaccine, but local and systemic reactions lasting 1–2 days are common with the inactivated parenteral vaccine. (See also Chapter 42.)

14.2.4. Antibiotic Prophylaxis. The success of preventing natural infection or disease or both with antibiotic prophylaxis depends on the sensitivity of the organism to the drug employed, whether single or multiple bacterial species are involved, the timing of administration in relation to infection, and the ability of the drug to reach effective concentrations in body sites before the organism is present. It has

Table 14. Prophylactic Uses of Antibiotics

Condition	Chapter (section) in this volume	Persons at risk	Antibiotic	Dose/time
Diphtheria	13(9)	Carriers of toxigenic strains	Penicillin or erythromycin	Full dose over 7–10 days
Gonorrhea	15(9)	Persons sexually exposed to infection	Penicillin	Full dosage as for treatment
Meningococcal meningitis	23(9)	Intimate contacts of cases or in closed outbreaks	Sulfadiazene for sensitive organisms	1 g adults or 0.5 g children q 12 h × 4 doses
			Rifampin	10 mg/kg per day for 4 days
Strep and rheumatic fever	34(9)	Rheumatic heart disease patients (prevention of rheumatic fever)	Penicillin (benzathine), i.m.	1.2 million U/mo
		Sometimes family contacts of strep cases	Penicillin, oral	200,000–250,000 U daily
Surgical infections	25(9.2.8)	Certain surgical patients[a]	Dependent on site of operation	
Syphilis	35(9)	Known exposures ("epidemiological treatment")	Penicillin	Same as treatment
Tuberculosis	39(9.3)	Recent skin test positive Contacts of cases Healed Tb patients; never treated Tb cases	INH	Adults 5 mg/kg per day for 12 mo

[a]C-V, C-section and vaginal hysterectomy, prophylactic hip, certain intestinal, biliary, CNS.

been employed in persons at high risk after known exposure in epidemics (meningococcus), in household contacts of cases (e.g., streptococcus, meningococcus, tuberculosis), and in sexual partners (gonococcus, syphilis), one of whom is infected. It has been employed after infection is diagnosed to prevent further spread or to limit complications (tuberculosis, rheumatic fever) or to limit the duration of the carrier state. The major limitations have been the development of antibiotic resistance, multiple organisms causing the disease, and poor patient compliance for long-term prophylaxis. Any mass prophylactic program aimed at a large group, especially a closed population, over a long term sets the stage for the development of resistance in the organism of interest as well as other circulating organisms. Antibiotic prophylaxis has met with debatable success when the bacterial sensitivity is not high, if the antibiotic is inhibitory but not bactericidal, if multiple organisms are involved (especially gram-negative), and if the risk of infection is relatively low, as in clean surgical operations. For greater detail, see books on clinical infectious diseases[38] or reviews on antibiotic usage,[138] as well as specific chapters in this book.

Table 14 lists some uses of antibiotic prophylaxis. The most successful are the prevention of recurrent streptococcal infections in persons with rheumatic heart disease and the prevention of disease in persons with infections due to *M. tuberculosis*, especially recent infections. Antibiotic prophylaxis with sulfadiazine for sulfa-sensitive organisms or rifampin for sulfa-resistant organisms is advocated for family or close contacts of patients with meningococcal meningitis or outbreaks in closed settings. These principles apply in large community outbreaks when sulfadiazine is given on a mass basis for sulfonamide-sensitive meningococci; of resistant strains in this situation, rifampin is not recommended because of the possibility of developing resistance to this drug.[17] "Epidemiological treatment" after known sexual exposure to gonorrhea or syphilis is effective, but requires a full therapeutic regimen. Prevention of infection during and after surgery is advocated in surgical procedures at high risk to infection such as total hip and knee replacement, colon–rectal surgery, and transurethral resection of the prostate.[139] Patients whose immune status is compromised by steroids, irradiation, alkylating and antimetabolic agents, and other immunosuppressive drugs are at high risk to certain bacterial, fungal, and viral organisms, including some that are not normally pathogenic, but no antimicrobial prophylaxis has been effective. They may be placed in protective isolation (see Section 14.2.1) and closely watched and antibiotic therapy instituted if infection occurs. A possible exception to the ineffectiveness of antibiotics in preventing infections is the prophylactic use of isoniazid in immunosuppressed patients with inactive tuberculosis. Antibiotics or other prophylactic measures for prevention of traveler's diarrhea are recommended only for the long-term traveler in high-risk endemic areas[80,106]; for short-term visitors, bismuth subsalicylate has proved useful.[43]

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CHAPTER 2

Public Health Surveillance

Philip S. Brachman

1. Introduction

The term *surveillance*, derived from the French word meaning "to watch over," may be defined as a system providing close observation of all aspects of the occurrence and distribution of a given disease through the systematic collection, tabulation, analysis, and dissemination of all relevant data pertaining to that disease.[1] The preferred term is *public health surveillance*, which emphasizes the focus of surveillance as discussed in this chapter to develop data that results in a public health preventive act ion. This distinguishes public health surveillance from other types of surveillance.[2] Although the methodology of surveillance is basically descriptive, its function is more than merely collective and archival. Surveillance must be dynamic, current, purposeful, and result in a public health action. This action frequently results in the establishment of a new or the reinforcement of an existing public health policy. It is fundamental for the prompt and effective control and prevention of disease. Traditionally, surveillance was first applied to the acute communicable diseases in the mid-1800s.[3] Since then, surveillance methodologies have been utilized to cover a many infectious diseases,[1] a wide variety of non-infectious diseases, and other health-related events such as environmental hazards, potential bioterrorism events injuries, vaccinations, the distribution of biological products, and health-care delivery.

2. History

William Farr, of the General Registrar's Office of England and Wales, is credited with initiating disease surveillance in the mid-1800s though mortality and some morbidity data were being collected during previous years in communities in other countries. He collected, collated, and analyzed vital statistical data and distributed reports to appropriate health personnel as well as to the public.[3] In the United States, the collection of national morbidity data was initiated in 1878, when Congress authorized the Public Health Service (PHS) to collect reports of the occurrence of the quarantinable diseases, namely, cholera, plague, smallpox, and yellow fever. In 1893, Congress passed an act stating that weekly health information should be collected from all state and municipal authorities. In 1902, in an attempt to develop uniformity, the Surgeon General of the PHS was directed to provide forms for collecting, compiling, and publishing surveillance data. In 1913, the state and territorial health authorities recommended that every state send weekly telegraphic summaries reporting the occurrence of selected diseases to the PHS. All states were reporting the occurrences of diseases by 1925. In 1949, when the National Office of Vital Statistics (NOVS) was established in the PHS, the communicable-disease-reporting function (morbidity reporting) was merged with the national mortality registration and reporting functions that were the primary responsibility of the NOVS. Until the early 1950s, the communicable disease reports were published weekly in the official journal *Public Health Reports*. When this journal became a monthly publication, the NOVS issued a separate weekly bulletin, the *Morbidity and Mortality Weekly Report* (MMWR), that was distributed to state health officers, state epidemiologists, county and city health officers, and others including persons who requested its receipt. In January 1961, the responsibility for receiving morbidity reports from the states and larger cities and issuing of the MMWR was transferred from Washington, DC, to the Communicable Disease Center [now called the Centers for Disease Control and Prevention (CDC)] in Atlanta, Georgia.

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In the United States, the application of the term *surveillance* to the watchfulness over a nationally important communicable disease (malaria) was begun in 1946 by the CDC[4] in part to monitor veterans who were returning from endemic areas. The benefit of applying a specific case definition and a critical epidemiological evaluation to the former rather than crude resulted in the recognition that malaria had ceased to be an indigenous disease. Endemic spread of infection had ceased some years before through control of the vector.

In 1955, following the initial introduction of the Salk, killed poliomyelitis vaccine, an epidemic of vaccine-associated poliomyelitis occurred ("Cutter Incedent"). The case was traced to one manufacturer's faulty manufacturing process. Subsequently, national surveillance of poliomyelitis was ordered by the Surgeon General as an essential step toward a solution for this national disaster.[5] In 1957, influenza was placed under surveillance in anticipation of the impending pandemic of Asian influenza for which a comprehensive national program of widespread vaccination and education of doctors and preparation of hospitals to meet such a possible disaster was undertaken by the Surgeon General. The influenza surveillance program has continued, and one of its essential functions is to provide information to guide manufacturers in the preparation of influenza vaccine as concerns its antigenic composition and the amount of vaccine to produce. In 1961, because of the increasing public health concern with salmonellosis, a special *Salmonella* surveillance program was developed in conjunction with the states to better define the problem so that appropriate control and prevention measures could be instituted. In recent years, surveillance has been extended to HIV, Lyme Disease, West Nile virus infection, SARS, avian influenza and many other public health illnesses or conditions.

The CDC has published a series of reports that further define public health surveillance. These include an outline of a comprehensive program,[6] "Guidelines for Evaluating Surveillance Systems,"[7] and "Case Definitions for Public Health Surveillance."[8] Responding to the reality of new emerging infections and reemerging infections, a number of surveillance systems have been implemented to define these diseases supported by CDC and other groups. Responding to reality, new surveillance systems are being developed and evaluated related to public health preparedness for noting as early as possible natural and man made disasters. There are several textbooks that have been published on public health surveillance (see Suggested Readings).

At present, the occurrence of 59 diseases is reported weekly and that of 7 other diseases are reported annually by state health departments to the CDC. Seven additional diseases are reported by either special case-reporting forms or line-listing forms submitted either monthly or annually. These reports are published in the MMWR and are summarized annually in the *MMWR Annual Summary*.[10] These lists are reviewed annually by the state and territorial epidemiologists and modified as indicated by the changing nature of the diseases and the occurrence of new diseases. Additionally, more intensive surveillance is maintained over selected diseases by means of special surveillance efforts to develop more specific data concerning these diseases. Selected non-infectious diseases are also under surveillance, such as birth defects, non infectious injuries, and diabetes mellitus.

National disease surveillance programs are maintained by most countries in the world. The methods used to report cases, the diseases to be reported, the analyses, and the type and frequency of reports vary, but the value and importance of surveillance is universally recognized. A number of European countries have been working to improve national surveillance programs and develop an European reporting system.[11] Desenclos has discussed various methods for collecting surveillance data.[12] The World Health Organization (WHO) maintains surveillance on the quarantinable diseases (cholera, plague, and yellow fever) as well as other selected diseases. The most recent disease to come under worldwide surveillance is acquired immunodeficiency syndrome (AIDS). WHO prepares weekly reports as well as other reports summarizing these data. WHO also responds to worldwide disease problems by developing special surveillance programs for emerging infections (i.e., Nipal virus, SARS) or for potential pandemic diseases (i.e., avain influenza).

3. Use of Surveillance

A surveillance program can be designed to produce a variety of output data depending on the purpose of the program. It can portray the natural history of the disease, including a description of the occurrence of the disease by time, place, and person. Surveillance data should describe the background (sporadic, endemic, or ongoing) level of the disease, as well as changes in the occurrence of the disease as modified by nonrecurring events such as epidemics. Surveillance can be used to monitor changes in the agent, such as antibiotic resistance in gonococci, staphylococci, pneumococci, and *Mycobacterium tuberculosis*, or changes in virulence, or genetic changes.

Analysis of surveillance data can help to establish priorities for developing a disease-specific control and/or a prevention program. Surveillance can also be used to confirm a hypothesis about the occurrence or distribution of

disease or indicate the need for further study or additional data. Analysis of surveillance data can lead to the development and/or institution of control and/or prevention measures such as chemotherapy, chemoprophylaxis, new resources or resource allocation (e.g., people, equipment, or monies), or additional training for persons involved in control and prevention activities. Surveillance can be used to evaluate the effectiveness of newly instituted control and/or prevention measures. Surveillance data are also important in forecasting or predicting the future pattern of the occurrence of a disease.

In this chapter, surveillance is discussed primarily as it involves bacterial infectious diseases. Surveillance techniques for other infectious diseases are similar, though there may be some variation in the data collection procedures.

4. Data Sources

The WHO in 1968 codified the term *surveillance* on a truly global basis.[13] Ten "elements" or distinguishable sources of data were identified Any one or any combination of the ten can be used to support a disease-specific surveillance program. The sources used to develop the surveillance data depend on the disease itself, the methods used for identifying the disease, the goals of the program, the personnel and material resources available, the population involved, and the characteristics of the disease's occurrence. One source of data can be used regularly and other methods utilized as necessary to improve the sensitivity and/or specificity of the data depicting the occurrence of the disease.

4.1. Mortality Data

Mortality registration has been used the longest, but it is useful only for diseases that are associated with fatalities. If the case-fatality ratio is too low, mortality statistics may not provide an accurate assessment of the occurrence of the disease. If mortality rate data are accurate and if the proportion of deaths to cases is known from past studies, then the number of deaths can provide an estimate of the actual number of cases that have occurred and define the pattern of disease occurrence over time.

Unfortunately, there is wide variation in the accuracy with which death certificates are filled out. Diagnoses are not defined consistently and diseases under surveillance may not be recorded even if they were a contributory cause of death, Also, there is a time lag in reporting deaths, so that a surveillance program based on mortality registration has an inherent delay of from weeks to months. In general, information derived from analyses of the cause of death on death certificates is inconsistent and likely inaccurate.

An example of the use of mortality data for surveillance is the collection of pneumonia and influenza weekly mortality reports from 122 American cities.[14] These data are used to describe weekly pneumonia and influenza activity and help determine the burden and geographic distribution of disease. Another example occurred in the 1960s during an investigation of shigellosis in rural areas in Central America where there was no ongoing surveillance program.[15] The only available reports of any of the cases were listings of deaths that were routinely noted in "vital statistics" books maintained in the communities. By noting the recording of deaths and by knowing the case-fatality ratio, it was possible to develop information concerning the occurrence of cases of shigellosis.

4.2. Morbidity Data

The second, and most commonly used, source of surveillance data is morbidity or case reporting. This is a prompt, simple, and useful system that is dependent on the reporting of cases of the diseases under surveillance. Reporting the occurrence of disease is the responsibility of the patient's health-care professional, usually a physician. He or she may delegate this responsibility to someone else such as a nurse, clerk, or administrator. Cases may be reported by the responsible person calling the health department or vice versa, or they may be reported each day or each week on a special form sent by mail or fax. Computers are also increasingly being used to report cases at all levels of reporting. Alternatively certain diseases lend themselves to mandatory reporting of laboratory results by laboratories, if the disease under surveillance requires a laboratory test for diagnosis. The techniques of reporting are described in greater detail in Section 6.

4.3. Individual Case Reports

Individual case investigation is more likely to be performed with rare diseases or unusual cases of a common disease. For diseases of high frequency, investigating individual cases is usually neither practical nor necessary, but may be conducted in order to obtain more specific case data or as a check on the validity of morbidity or mortality reporting. As a disease decreases in incidence, individual case investigation may be of increasing importance to determine why the case occurred and to further direct control and prevention measures. As the occurrence of a disease decreases, leading toward prevention, elimination, or eradication status, then intensive investigation of each reported case is important. The value of this approach was dramatically demonstrated

in the smallpox eradication program[13] and is currently being practiced in the international poliomyelitis eradication program.[16] Additionally, as measles immunization activities are being intensified in the United States, individual case reporting is being emphasized.[17]

4.4. Epidemic Reporting

The fourth source of surveillance data is the reporting of epidemics. Frequently, there is quantitative improvement of reporting when clusters of cases occur. Thus, single cases of shigellosis or salmonellosis may not be individually reported, but if there is an epidemic, then all cases that are part of the epidemic may be reported.

4.5. Epidemic Field Investigation

Epidemic field investigations may uncover more cases of the disease than would have been reported without the investigation. In the epidemic of salmonellosis in Riverside, California, in 1965, several hundred cases were initially reported; however, following a field investigation, 16,000 cases were estimated to have occurred.[18] In 1987, an epidemic of *Salmonella typhimurium* gastroenteritis occurred in Chicago, Illinois, related to pasteurized milk and 16,000 cases were cultured positive for the organism. However, community surveys suggested that between 170,000 and 200,000 cases occurred.[19] In 1993, cases of cryptosporidiosis were reported from Milwaukee, Wisconsin. Contaminated, inadequately purified city water was shown to be the source of infections. Subsequently, investigations suggested that more than 400,000 cases had occurred. The decision to investigate an epidemic will be based on the specific disease, the seriousness of the outbreak, the extent of the problem, the anticipated need for more specific information concerning the occurrence of the epidemic, availability of resources, research potential, and possibly political pressures.

4.6. Laboratory Reporting

The laboratory is essential in identifying and confirming pathogens. Although many diseases can be adequately described clinically, there are others for which laboratory identification of the etiologic agent is essential for accuracy. For example, gastroenteritis may be caused by various organisms; but it is frequently not possible to be certain of the etiology on the basis of clinical and epidemiological data alone. Thus it is necessary to perform laboratory testing to identify the etiologic agent. The accuracy of the *Salmonella* and *Shigella* surveillance programs in the United States depends on laboratory testing. Every state now has laws that identification of agents of reportable infectious diseases must be reported to the local health authorities.

In addition to disease identification, the laboratory can also provide important information concerning specific characteristics of microorganisms that are epidemiologically important. For example, the antigenic characteristics of influenza strains are important, since significant changes in the prevalent strain will necessitate changes in formulation of vaccine to be used before the next influenza season. An example is the surveillance of avian influenza strains isolated from avians and humans to identify genetic changes that will increase the likelihood of human-to-human transmission. Identifying the serotype of salmonellae isolated from different patients may be necessary in order to associate different isolates as part of a single epidemic and to identify the source of infection. Careful attention to antibiotic sensitivity patterns can indicate a change in the epidemiological pattern of the disease or may be a forewarning of an impending upsurge in the occurrence of the disease. This has been seen in the increasing frequency with which antibiotic-resistant gonococci are being identified,[20] the spread of methicillin-resistant *Staphylococcus aureus*[21] within and between hospitals, and the increasing identification of vancomycin-resistant enterococci as well as of multiresistant *M. tuberculosis* (see Chapters 15, 33, 25, and 39). A variety of molecular tools are now available that provide highly specific identification of a strain or substrain of an organism. Methods for both phenotyping and genotyping are now used. Techniques such as the polymerase chain reaction (PCR), genome sequencing, immunoblot electrophoresis, and a variety of DNA and RNA probes have given high sensitivity and high specificity to the detection of organisms in various tissues and to the precise identification of the particular strain causing a given epidemic. Highly specific antibody identification is also now possible through commercially available monoclonal antibodies. New emerging technologies, such as proteonics and others, are adding to our ability to further identify pathogens (see Chapter 3). These techniques make it possible to follow the spread of an organism in an epidemic and to differentiate between exogenous reinfection and endogenous reactivation.

The serology laboratory also contributes to surveillance by identifying and/or confirming the presence of a specific disease. Usually, two serum specimens are obtained from each individual, one during the acute phase and one during the convalescent phase of illness, to demonstrate a significant change (usually fourfold) in titer. However, if only a single serum specimen is obtained, the occurrence of a specific disease may be suggested if the antibody titer to that disease is

elevated beyond a certain value or by the presence of IgM antibody. Also, if elevated titers are found in serum specimens from a group of patients who had similar illnesses, then these single specimens can be of assistance in making the diagnosis (see Chapter 1).

4.7. Hospital Reporting

The reporting of infectious diseases among hospitalized patients, previously hospitalized patients, or outpatients is a valuable and important source of information. These infections may be truly hospital acquired (nosocomial) or community acquired (see Chapter 26). Community-acquired infections among patients admitted to a hospital reflect the occurrence of infectious diseases in the community. Infections that develop among hospitalized patients or outpatients may represent an endogenous or a exogenous source of infection. If exogenous, the source may be another patient, hospital employee, rarely a visitor, or the hospital environment. An exogenous source may reflect a community problem. Endogenous infections reflect organisms from the patient who may have become colonized from a community source. Computerized administrative data systems can strengthen the hospital surveillance program (see Chapter 26).

4.8. Surveys

Surveys can provide information concerning the prevalence of disease. Clinical surveys may include questions related to the occurrence of a disease, physical examination such as spleen surveys to identify patients with malaria, or diagnostic tests such as skin tests to determine the prevalence of histoplasmosis or tuberculosis. In some countries, blood smear surveys may be used in surveillance for malaria. Other types of surveys include household surveys, such as the National Health Interview Survey, which includes 55,000 households surveyed annually; cluster surveys, such as are used in evaluating immunization programs; and telephone surveys, which can be used to estimate the magnitude of an outbreak of a disease.

Serological tests for certain bacterial, rickettsial, and treponemal infections carried out on a representative sample of a population can provide prevalence data for different age, sex, and geographic segments of the group tested. Incidence data can be obtained by demonstrating the appearance or rise in antibody titer to a given infection in two serum specimens spaced in time such as the start and end of an epidemic, military service, or a college year; the occurrence of recent infection can also be demonstrated in a single specimen by determining the presence of specific IgM antibody for that infectious agent or antibody reflecting early infection. The uses of seroepidemiology are presented in more detail in the companion book on viral infections.[22]

4.9. Animal Reservoir and Vector Distribution

Animal reservoir and vector distribution studies are important in maintaining surveillance of zoonotic and arthropod-borne diseases. Information about rabies in animal reservoirs in a specific geographic area has been and remains important in making a decision concerning the need to treat a human exposed to an unidentified animal. The knowledge that tularemia is occurring in animals or that ticks infected with *Francisella tularensis* are present in an area would support reports of suspect cases of tularemia in humans. Similar studies are in progress to define the distribution of Lyme disease, which is caused by *Borrelia burgdorferi* whose vector is one of several Ixodid ticks. Knowledge about the occurrence of plague in prairie dogs or rodents can be important in evaluating data concerning possible cases of human plague. Surveillance of animal rabies is important as a warning system for the potential occurrence of human rabies. Monitoring the occurrence of avian influenza in birds has been important as concerns the development of influenza in humans due to infections with the avian influenza virus.

4.10. Biologics and Drug Distribution

The utilization of biologics and drugs for treatment or prophylaxis of a disease may be used to monitor disease occurrence. For example, in an outbreak of diarrheal disease, the increasing sales of antidiarrhea medications by pharmacies serve to corroborate the occurrence of disease as occurred in the epidemic of cryptosporidiosis in Milwaukee in 1993.[22A] Similarly, an increase in requests for immune serum globulin can be a clue to the occurrence of cases of hepatitis A.

4.11. Demographic and Environmental Data

Demographic and environmental data are necessary to analyze disease occurrence data effectively. Such data may include age, sex, occupation, residence, or other personal information. Incidence rates cannot be determined until denominator data concerning the population are available. For example, when an increase in the number of isolations of *Salmonella eastbourne* was noted, an analysis of cases by age showed a significant number of cases among young children.[23] This fact was an important clue in leading the investigators to identify chocolate candy as the vehicle of

infection. The ability of new molecular and electrophoretic techniques to identify the specific strain of organisms such as *Salmonella* has allowed epidemics in different geographic areas to be interlinked and a common commercial source identified.[24] The problem of multiple drug resistant tuberculosis in New York City prisons is an example of antibiotic sensitive testing showing the relationship of these cases that occurred in a number of prisons (see Chapter 40).

4.12. News Media

Another useful source of surveillance information is public information gathered through the news media. It is not uncommon for the occurrence of a disease, and especially an epidemic, to be first noted by the news media. Additionally, the news media can perform an important role in alerting the public to the occurrence of a disease epidemic, and thus stimulate the reporting of cases that otherwise might not have been diagnosed or reported. In an epidemic of botulism associated with a restaurant in a western state, radio reports alerted a patron of the restaurant to the possibility of exposure after the patron had returned to his home several hundred miles from the restaurant.[25] At the time he heard the radio report, he was experiencing some symptoms. Accordingly, he sought medical assistance, botulism was diagnosed, he was successfully treated, and another case was reported. The national epidemics of *Escherichia coli* 0,157:H7 in spinach and salmonella in peanut butter are other examples of the media in surveillance.[25A,25B]

5. Routine Surveillance

As previously indicated, routine surveillance of a specific disease will not include all the data sources discussed above. The methods that provide the most accurate information collected in a practical and efficient manner that satisfies the objectives of the surveillance program should be utilized. If more information is needed concerning occurrence of the disease, then additional sources of information can be incorporated into the surveillance system.

The need for completeness of reporting varies according to the incidence of the disease under surveillance. For those diseases that either normally do not occur in an area or occur at a very low incidence, it is essential, for control purposes, that all cases be reported. Examples (in the United States) include plague, yellow fever, poliomyelitis, human rabies, measles, hanta virus disease, avain influenza, and agents of boterrorism.

On the other hand, to maintain surveillance on diseases that commonly occur, it is not critical for all cases to be reported. In the United States, it is estimated that only 1% of cases of salmonellosis, for example, and 15–20% of cases of viral hepatitis are reported. The fact that all cases are not reported should not reduce the effectiveness of surveillance, since it is generally the trends and patterns of disease occurrence that are important in the detection of problems and implementation of control and prevention measures. Changes in the trend should reflect real changes in the occurrence of disease and not changes reflecting a variation in the methods of surveillance. If the methods of obtaining the surveillance data have not changed significantly during a period of time, the case definition has not changed, and the data collected are a representative sampling of the cases that have occurred, then these data should be suitable for determining the trend of the disease. However, a change in the methods used to collect the surveillance data, followed by a change in the reported occurrence of the disease, may be falsely interpreted as a change in the incidence of the disease. An example of this artifact could have occurred in the national *Shigella* surveillance program that was initiated in 1964, with routine reporting from 17 states. Several years later, the remaining states began reporting their isolates; the sudden increase in reported cases could have been misinterpreted if attention had not been given to the mechanics of the surveillance program.[26] Similar artifacts in surveillance data have occurred in the national AIDS surveillance program. As the definition of a case of AIDS has been modified to accommodate new knowledge concerning the clinical manifestations of the disease, the overall surveillance data have reflected these changes.[27]

Case reporting of certain diseases may be discontinued because effective control measures are not available. One such example is streptococcal infections, which may give the uninformed the wrong idea that these infections are no longer of importance.

To validate surveillance data, various methods have been used. These include (1) active surveillance, that is, physicians in the reporting area can be called and asked if they reported all cases of the disease among the patients within a specific time period; (2) hospital records can be checked by means of a prevalence study to see that all notifiable diseases have been reported; and (3) laboratory reports from hospital and public health laboratories for a given disease can be compared to the cases reported to the health department.

6. Reporting

In general, detailed individual case data are not necessarily useful in surveillance programs. It is the analysis of

collective data that provides meaningful information. If more specific case information is necessary, then individual cases can be traced back and additional data obtained.

The quality of a surveillance program is as good as the quality of the data collected. In morbidity reporting, an integral component is the person who has the responsibility for reporting the occurrence of the disease. Most frequently, this is the person who has medical responsibility for the patient, and usually that person is a physician. This responsibility may be delegated to someone else, such as the physician's nurse or, in a hospital, infection control practitioner, the house staff or the administrator. The increasing use of computers by hospitals and large health maintenance organizations (HMOs) for recording diagnoses and the development of linkage systems for such data provide new means of collecting and evaluating surveillance data. In reporting cases of any disease by any means, confidentiality of the patient must be respected and maintained. The Health Insurance Portability and Accountability Act (HIPPA) law concerning patient confidentiality does allow the reporting of patient data but emphasizes the need to maintain confidentiality of the patient (Ref).

In the United States, every state has a law indicating the occurrence of which diseases must be reported and the mechanism of how they should be reported and to whom. Additionally, there usually is a penalty assessed, a monetary fine, prison time, or demerit system that will prohibit renewal of medical license of a reportable case is not reported. However, it is not apparent that any penalty has ever been assessed. The reporting from the States to CDC is a voluntary system and as previously stated the diseases to be so reported are discussed and subject to be modified each year as discussions between the CDC and the state epidemiologist. Within a community, cases are reported to the local health authority, such as a city health department. At regular intervals, usually weekly, these cases will be reported to the state health department. In the United States, the weekly totals and selected individual case data will be reported to the CDC. Many local health departments report to the state health department by computer. All state health departments report their data to the CDC via computers. The development of worldwide systems of communication such as the Internet and World Wide Web has yielded a global method of making such data widely available. The MMWR and the *WHO Weekly Record* are available in these systems. Computer reporting by health-care physicians has been initiated in France[29] and in China.[30]

6.1. Motivation

The diligence with which cases are reported reflects the motivation of the person responsible for reporting. Physicians frequently do not wish to assume this responsibility because of the constraints on their time and the low priority they give to reporting. Also, they may not be aware of state law that mandate reporting of specific diseases. They may not know how to report. There needs to be some motivation developed for reporting other than that related to disease-reporting laws. Motivation may result from being a participant in a public health project or from professional or personal gain. A report summarizing the surveillance data (see Section 9) may be motivational (as well as educational), so that the reporter does recognize that some action results from disease reporting. Motivation may also be derived from knowledge that surveillance data can support the development of effective control and prevention programs, with a decreased incidence in the occurrence of disease.

Reporting may be stimulated by the availability of an epidemiologist to provide assistance to the reporting physician on request. Another source of motivation may be that the reporting of surveillance data results in important clinical and therapeutic data being made available to the practitioner. For example, the increase in antibiotic-resistant strains of *Neisseria* gonococci reported through the CDC's surveillance program is important information for practicing physicians to have when they see a patient, make a clinical diagnosis of gonorrhea, and want to initiate therapy immediately. Another example is the increasing number of reports of antibiotic-resistant *Mycobacterium tuberculosis*, which is a significant problem to clinicians as well as to public health professionals. Also, the worldwide problem of resistant strains of the pneumococcus has been another serious situation as is the problem of the increasing number of reports of community-acquired multiple-resistant *Staphylococcus aureus* (MRSA) (see Chapter 34), the increasing resistance of Neissaria gonorrhea (see Chapter 16). These examples of the application of surveillance data to the practice of medicine can serve to motivate physicians to participate in surveillance activities. Disease reporting can also be stimulated by making specific therapeutic drugs available to the physician on notification of the occurrence of a specific disease. In some communities, reports of hepatitis A result in serum immune globulin being made available for prophylactic use. A report of a case of botulism may lead public health authorities to make botulinum antitoxin available. Reports of certain tropical parasitic diseases make it possible for physicians to obtain certain therapeutic drugs from the CDC not otherwise available. The availability of a drug exclusively from the CDC in the early 1980s to treat cases of *Pneumocystis carinii* pneumonia led to recognition of a new disease, AIDS, in 1981.[31]

A reward system for reporting can be used. The reward can be publicity given to the reporting physicians by listing their names in the surveillance report or in a scientific paper

summarizing the surveillance data. A monetary reward system has also been tried, with an annual payment or a specific amount of money being given for each report submitted. This activity was not very successful, however, and is no longer being practiced.

Health officials must recognize the negative effect of a reporting mechanism that is too complex or that demands excessive expenditure of time on the part of the reporter. If reporting of cases brings adverse publicity to the patient, physician, hospital, or community, surveillance will be inhibited. Adverse publicity that leads to a loss of money or to legal action against the reporter or hospital also has a negative effect on the reporting of disease. Some countries choose not to report quarantinable diseases to the WHO because of the knowledge that publicity concerning the occurrence of those diseases may have an adverse effect on the movement of people and goods across their borders.

6.2. Ease of Reporting

To stimulate reporting, the mechanisms must be simple and yet compatible with an effective and sensitive surveillance system. There must be a relatively easy mechanism by which cases can be reported to the public health authorities. However, the information being requested must provide adequate data for developing a meaningful and practical control and prevention program. It is important to request only data that meet the objectives of the surveillance program. If superfluous data are requested and collected but not used, the reporter will question the surveillance effort and support for the program will decline.

Reporting from the health-care practitioner to the local health department could be simplified to the extent that when the diagnosis of a reportable disease is made and entered into the health-care providers' computer that can be programmed to automatically report the case to the local health department. Only the patients' data that should be reported would be provided to the local health department. Additionally, the local health department's computer can be programmed to report the reportable diseases, either in singularly or in batches, to the next level of reporting (regional, district, and/or state). Thus, all reporting from the health-care provider (or laboratory) to CDC can be accomplished by computers.

6.3. Case Definition

It is important in developing a surveillance program that *specific* case definitions be developed and publicized so that those persons participating can accurately report cases. The definition must be simple, acceptable, and understandable and not incorporate diagnostic criteria that are difficult to comprehend. If laboratory test results are part of the definition, the test must be readily available and inexpensive and not demand a great deal of the patient. It is also important to consider whether only confirmed cases should be reported or whether reporting should also include cases that are less definite as cases of the specific disease and are classified as presumptive or suspect cases of the disease; if so, the definitions of these categories must be acceptable and publicized. The CDC has developed and published a compilation of case definitions for public health surveillance that standardizes definitions for all the diseases reportable in the United States.[8]

6.4. Passive Reporting

Surveillance reporting may be passive or active. Passive surveillance is the routine reporting in which case reports are initiated by the health care provider. Preprinted postcards or reporting forms and stamped envelopes routinely supplied to the reporter can be used to report the requested surveillance data. These cards can be mailed individually or at weekly or monthly intervals, summarizing all cases seen during that time interval; negative reports, i.e., the lack of occurrence of cases, can also be requested. Some states divide the reportable diseases into those commonly seen and those only rarely seen. The reporting official is asked to provide a negative report if no cases of the common diseases are seen and to fill out the form for a rare disease only when a case is actually seen. The reporting form can be a general form suitable for a number of diseases or a specific form used only for a single disease. Special forms can be developed if more detailed data are desired.

Reporting to the local public health office may be done by telephone. The report may be taken by a clerk during working hours or tape-recorded if phoned in outside working hours. The tape recording can be subsequently transcribed by a clerk, who can call the physician if more data are required. To stimulate reporting from throughout an area such as an entire state, a toll-free telephone system can be established. It has been demonstrated that the availability of an automatic telephone-answering service for use at any time has stimulated reporting of disease by physicians. Passive reporting can be improved as health-care providers better utilize personal computers in their practice. This could stimulate better and more rapid reporting and decrease the error rate due to mishandling data.

6.5. Active Reporting

An active surveillance system can be instituted to improve the opportunities to obtain surveillance data; it can

be used for routine surveillance or be an integral part of a special surveillance program established to monitor a specific disease such as during an epidemic. In active surveillance, public health officials needing the information contact the reporter at regular intervals and specifically ask about the occurrence of the disease(s) under surveillance. Thus, there is an active attempt by public health officials to obtain disease occurrence information from the reporter. The introduction of an active surveillance system may greatly increase the number of reported cases of a given disease and may even simulate an epidemic. The users of the surveillance data must be thoroughly informed of such changes in surveillance methods and an interpretation of the increase discussed in an editorial comment. While this system increases the sensitivity of reporting, it also increases the cost of surveillance due to the time needed to make active contact with the reporter.[32]

6.6. Sentinel Physician Reporting

A sampling system that can incorporate either active or passive surveillance is known as a sentinel reporting system. Depending on the size of the community, the degree of reporting desired, and the disease(s) under surveillance, the sentinel physicians may be a sample drawn from all practicing physicians or from among certain specialists who are more likely to see cases of the disease under surveillance. If a patient with the disease under surveillance may be seen by any physician, then the sample should be drawn from among all practicing physicians; however, if a childhood disease is under surveillance, then pediatricians and family practitioners would be the group from which the sample is drawn. The same sentinel physicians can be requested to report regularly, or alternating sentinel physicians can be selected to report weekly or monthly. If the reporting physicians were selected randomly, then the reported cases may be extrapolated to project the total number of cases of the disease that occurred in the area covered by the physicians, weighing each physician's reported cases according to the size of his or her practice. This will allow a fairly accurate estimate of the total cases to be made. However, if the sentinel physicians were not randomly selected or if they were volunteers, the total number of cases can only syndromic reporting be approximated from the number reported.

6.7. Laboratory Surveillance

Disease surveillance can also be maintained by regular monitoring of laboratory reports for the identification of etiologic organisms for diseases under surveillance. This system may be of secondary importance in that it serves to confirm a clinical diagnosis, or it may be of primary importance in identifying the etiology that was suspected by the clinician. For example, a case of pulmonary tuberculosis can be fairly accurately diagnosed on the basis of history and clinical evidence, including radiographs and a positive skin test. Identification of the organism in sputum confirms the diagnosis. Additionally, the antibiotic sensitivity of the organism can be determined that will confirm the proper treatment regime. However, with some diseases such as salmonellosis or syphilis, or shigellosis, accurate diagnosis is dependent on the laboratory identification of the etiologic agent. In diseases in which the laboratory plays a key role in identifying the etiologic agent, it is important to use the appropriate media necessary for this identification. For example, in maintaining surveillance for *Vibrio cholerae* in the United States, it is necessary to use special plating media such as thiosulfate-citrate-bile salt-sucrose (TCBS) agar if the organism is to be identified. Another example is the recognition of the importance of *Yersinia enterocolitica*. When 3 weeks of cold maintenance was required for culturing the organism, very few cases of diarrhea due to this organism were identified. With the introduction of a new culture medium (CIN) that eliminated cold storage, rapid identification became possible and cases are now being identified in routine laboratories. DNA/RNA probes and other microbiological molecular methods of diagnosis are becoming increasingly available and used.

Antibiotic-resistant microorganisms have become an increasing serious problem making it necessary for laboratories to perform antibiotic sensitivity testing as appropriate. These data must be reported to the health-care practitioners in the area in order for them to provide the proper therapy.

6.8. Hospital Surveillance

Surveillance can also be maintained by using hospital records (inpatient or outpatient) and hospital databases to detect either hospital-acquired or community-acquired infections. The records can be abstracted by specially trained enumerators or by record-room personnel (see Section 7.7 for further discussion of hospital surveillance, as well as Chapter 26).

6.9. Absenteeism Surveillance

Other methods of obtaining surveillance information depend on the specific disease. For diseases with high morbidity, an effective surveillance program can be developed by noting absenteeism from schools or industry, depending

on the ages of the involved population. All absenteeism during the period under surveillance may not reflect cases of the disease, so that further information may be needed, such as the rate of absenteeism due to other causes. Sickness benefit or insurance claims can also be utilized to develop surveillance data.

6.10. Syndromic Surveillance

Syndromic surveillance is the reporting of disease by the syndrome's signs and/or symptoms and not its etiologic diagnosis. This form of surveillance is commonly in place in developing countries in which physicians or academically trained health-care providers are not located in health clinics but there are health aides who can report. This form of surveillance can portray disease trends for purposes of control and prevention.

With the concern about bioterrorism, syndromic surveillance is being evaluated as an early warning system following the release of a biological agent.[32A,32B]

7. Special Surveillance

Special surveillance efforts can be established when the rate of occurrence of a disease increases either as part of the expected trend of that disease (periodic or seasonal increase) or as an unusual increase (epidemic). Special surveillance programs can also be developed in relation to the identification of a new disease entity; to provide disease data for research or investigation projects; to define the population among whom prevention measures should be instituted, such as vaccination; and also to evaluate control and prevention measures. Once the immediate need for a special surveillance system has been accomplished, the system should either be stopped or a regular surveillance program for that disease should be developed. In 1981, when the first cases of AIDS were reported, a special surveillance program was initiated. Once the national importance of surveillance data was identified, a regular surveillance program was established. All states now routinely report cases of AIDS to the CDC as part of the weekly surveillance program.

7.1. Influenza

Surveillance information concerning influenza is developed from the regular weekly reporting by 122 American cities of deaths from pneumonia and influenza. Reports of outbreaks of respiratory disease and reports of virus isolation from laboratories doing routine diagnostic work also provide surveillance data. To complement this system, at the beginning of the anticipated influenza season, additional surveillance activities are initiated to improve the knowledge concerning the occurrence of the disease.[33] Reports of absenteeism are solicited from selected industries and schools, since increased absenteeism may be one indication of increased influenza activity. Sentinel family practitioner volunteers from throughout the country report the percentage of patients they see in their practice with the clinical diagnosis of influenza. Virus laboratories are encouraged to process additional specimens from patients with symptoms of a respiratory disease, in order to increase the opportunity of isolating influenza viruses and subsequently to report the results to public health authorities. New rapid laboratory methods now facilitate recognition. These special surveillance activities are helpful in increasing the sensitivity of surveillance for influenza. With the advent of avian influenza in Asia, Africa, the middle east, and southern Europe, involving avians and humans, the intensity of surveillance has increased significantly. Countries are urged to report to the World Health Organization human and avian cases as soon as they are diagnosed and to make isolated influenza virus strains available to WHO for potential use in vaccine production. All such strains are being monitored for antigen drift or shift, which is important not only for vaccine formulation, but also to identify a new strain that may have greater ability for human to human transmission.

7.2. Gastroenteritis

During an epidemic of a gastrointestinal illness, such as salmonellosis, special surveillance efforts of a particularly high-risk group can be introduced to improve the information developed concerning the occurrence of the disease. This may include morbidity reporting, laboratory surveillance, case reporting, or field investigations. With these extra data may it be possible to recommend the most appropriate control and prevention measures. CDC initiated PulseNet in 1996 as an international network of public health laboratories that perform subtyping on bacteria that may be foodborne. Currently, the subtyping method is standardized pulse-field gel electrophoresis, with the results compared electronically with a database maintained by CDC. There are thousands of patterns from throughout the world on file. CDC also developed FoodNet in 1996 with the objectives of determining the burden and trends of foodborne illness in the United States. This surveillance program is able to relate illness to specific foods and to assess interventions. This program is a collaboration with the United States Department of Agriculture, the Food and Drug Administration, and 10 participating state health departments that include more than 650 clinical

laboratories. It receives reports of the isolation of nine enteric pathogens (see Chapter 6).

7.3. Guillain–Barré Syndrome

During the swine flu vaccination program (1976), the reported occurrence of Guillain–Barré syndrome among vaccinated persons resulted in the development of a separate, active, surveillance effort involving special reports from neurologists. Surveillance subsequently has failed to associate other influenza vaccines with this reaction.

7.4. Reye's Syndrome

To develop information concerning the possible association of Reye's syndrome with influenza, special surveillance activities and reporting forms were established for use by pediatricians and neurologists, who were more likely than other physicians to see patients with Reye's syndrome.[35]

7.5. Infant Botulism

When the first reports of infant botulism were published, it was apparent that a previously unrecognized public health problem needed defining. Accordingly, a special attempt at developing surveillance data was initiated by alerting public health officials as well as pathologists, pediatricians, and laboratories to the disease entity.[36] A special reporting form was developed so that pertinent information could be obtained on each case. These special efforts resulted in the reporting of additional cases and the accumulation of important epidemiological data that helped to improve our knowledge of the disease. Cases of this disease are now reported as part of the routine surveillance program in the United States.

7.6. Legionnaires' Disease

When this disease was first identified, there were many unsolicited reports of possible cases. In an effort to standardize these data to define the clinical entity better and to develop more useful epidemiological data, a special surveillance reporting form was developed and a specific definition of a case of Legionnaires' disease was publicized.[37] This resulted in important, new data being uncovered concerning this newly described disease entity. Cases of this disease continue to be reported as part of the routine national surveillance program.

7.7. Hospital Infections

Because of the increasing problem of hospital-acquired infections (nosocomial infections), special surveillance efforts have been developed within hospitals to accumulate data that might be useful for instituting procedures to control and prevent nosocomial infections.[38] The development of infection control committees with many responsibilities, one of which is to supervise a nosocomial infection surveillance program, has been of prime importance to these surveillance programs. Special personnel are usually dedicated to this activity. Surveillance data can be collected by various techniques including individual reports from physicians or floor nurses, ward rounds and record reviews by the infection control nurse, and regular review of the laboratory and pathology records. During ward rounds, the infection control nurse seeks clues to infection by asking physicians and nurses whether any of their patients have infections and noting which patients have elevated temperatures, are receiving antimicrobial agents, or are in isolation. The outpatient department and the employee health service can also be kept under surveillance, since infections noted in these areas may reflect infections among inpatients. All these sources of nosocomial infection surveillance data may not be incorporated into the routine surveillance program at the same time. Also, surveillance may be targeted at specific high-risk patients, areas, particularly, intensive care units, or procedures. As with general surveillance programs, the methods incorporated should reflect the specific needs of the program and available resources. Computer-based surveillance has been developed and may utilize hospital databases such as the admission, transfer, and discharge database to track patient movement, the laboratory database to identify particular infections or antibiotic resistance, and the pharmacy database to identify patients receiving antimicrobics. Community-acquired infections may also be brought under surveillance as an addition to the hospital infection surveillance program. The surveillance and recognition of hospital infections are dealt with in greater detail in Chapter 26.

7.8. Arbovirus Diseases

CDC has developed a special surveillance program for arbovirus diseases, called ArboNet, an Internet-based surveillance system managed by state health departments and CDC. This system has been valuable in tracking the spread of West Nile virus infection and diseases from the northeastern United States in 1999 to all contiguous states by 2005.

7.9. High-Risk Population

A special surveillance program can be developed to identify specific disease among high-risk populations who will derive the greatest benefit from a particular prevention measure. For example, this has been useful for both meningococcal and pneumococcal disease for which specific, effective vaccines have been developed and are now recommended for use among specific highly susceptible populations that were defined from analysis of surveillance data. The administration of influenza and pneumococcal vaccine to particular groups has been mandated by the Centers for Medicare and Medicaid (CMS) and computer-based programs have been introduced to vaccinate target groups and prevent lost opportunities for prevention.

7.10. Emerging Infections

The recognition of AIDS in the United States in 1981 raised the question of whether our surveillance systems were adequate to recognize new diseases caused by agents such as Lassa, Ebola, and Marburg viruses, as well as other older agents that were mutating, such as influenza, or the occurrence of antibiotic resistance to common infectious agents such as the pneumococcus, tubercle bacillus, and enterococci. This led to a National Institutes of Health (NIH) conference in May 1989 that dealt with emerging viral infections, their development, surveillance issues, and means of recognition, which has been subsequently published in a book edited by Morese.[39] In June 1991, the Institute of Medicine (IOM) of the National Academy of Sciences convened a 19-member committee to review the broader field of all emerging infections. This report was published in 1992 and discussed six factors that the committee felt could account for the occurrence of emerging and reemerging infections.[40] Their report also discussed some actions including surveillance that could help control these problems. Another IOM committee was named in 2003 to bring these discussions up to date. They extended the list of factors to 13 and stressed the need for new novel surveillance systems to better define the problems.[40A]

The CDC was quick to recognize the need for additional methods for surveillance and control of these infections.[41] The CDC has developed Centers for Excellence for Public Health Information at five universities, part of whose mandate is to develop novel systems of surveillance and to incorporate electronics into surveillance systems. The role of the United States in emerging infections has been defined by Henderson.[42]

7.11. Serosurveillance

The use of serological testing on a regular basis may provide disease occurrence information not available by other less costly methods. A reproducible serological test and a responsive, approved laboratory are necessary ingredients for this activity. Serology may be useful in adding information to regular surveillance methods and reflects clinical and subclinical infections. Another example of the use of serology is seen in communities in which there is an interest in determining the incidence of human immunodeficiency virus (HIV) infections among pregnant women for which a serosurveillance program is developed in order to provide the necessary data. Serosurveillance has also been used to track the movement of West Nile virus activity across the country.

Other special surveillance techniques can be incorporated to handle specific problems. For example, if there is concern regarding foodborne diseases, then in addition to the routine reporting of cases and epidemics, surveillance of potentially contaminated foods can be instituted by utilizing laboratory culturing of foods routinely supplied by commercial sources. The importation of food items from abroad and their nationwide distribution has resulted in interlinked geographically distant epidemics of the same infection. This requires closely collaborative surveillance activities in state health departments working through the CDC and with the Federal Department of Agriculture (FDA). Surveillance of human carriers of certain pathogenic organisms such as staphylococci or salmonella can be initiated if there is concern about the occurrence of disease transmitted by asymptomatic carriers.

8. Data Analysis

Once the data have been collected, they must be collated and analyzed at regular intervals. The analysis can be simple or complex depending on the needs of the surveillance program, time constraints, how the data are to be used, and the personnel and facilities available. In addition to analyzing data in the routine manner (cases per 100 population), other methods of quantitating disease occurrence data are being utilized for some diseases. These methods provide a more realistic appraisal of the impact of the disease's occurrence upon a population. Some of these methods include calculating years of potential life lost (such as in evaluating injuries), number of cases of a disease per exposure day (such as ventilator-dependant pneumonia per ventilator days), and number of cases of a disease per procedure performed (bacteremias per cardiac catheterization). As surveillance becomes more complex and as more data are

handled, computerization of the data may be desirable and necessary. As previously stated, surveillance data can be entered directly into the computer by the reporting office instead of being handled by one or more intermediate individuals. There are software programs available that can analyze the surveillance data and prepare figures summarizing these analyses. One such software program is Epi Info which is used by the CDC to handle the data reported through the country's disease surveillance program, including preparing the data for publication in the MMWR.

Data analysis will usually suggest the best point for intervention. Occasionally, additional data may need to be gathered by additional surveillance activities or by special investigations.

8.1. Frequency of Review

The frequency, type, and complexity of the analyses are dependent on the use of the summary data. A routine surveillance program may require analyses at monthly or weekly intervals; in epidemic circumstances, it may be necessary to review the surveillance data at more frequent intervals, such as weekly or even daily. The data should be analyzed according to the three key epidemiologic variables, time, place, and person.

8.2. Time

When characterizing the data by time, there are four trends to consider. The first is the *secular* trend, which refers to the occurrence of the disease over a prolonged period of time, such as years. The secular trend of tetanus is one of gradually decreasing incidence (Figure 1). The decreasing secular trend of an infectious disease can be the result of the interaction of various factors such as socioeconomic educational, nutritional, and specific and nonspecific immunity factors within the involved population.

The *periodic* trend,which is the second time trend to consider, refers to the temporary variations from the

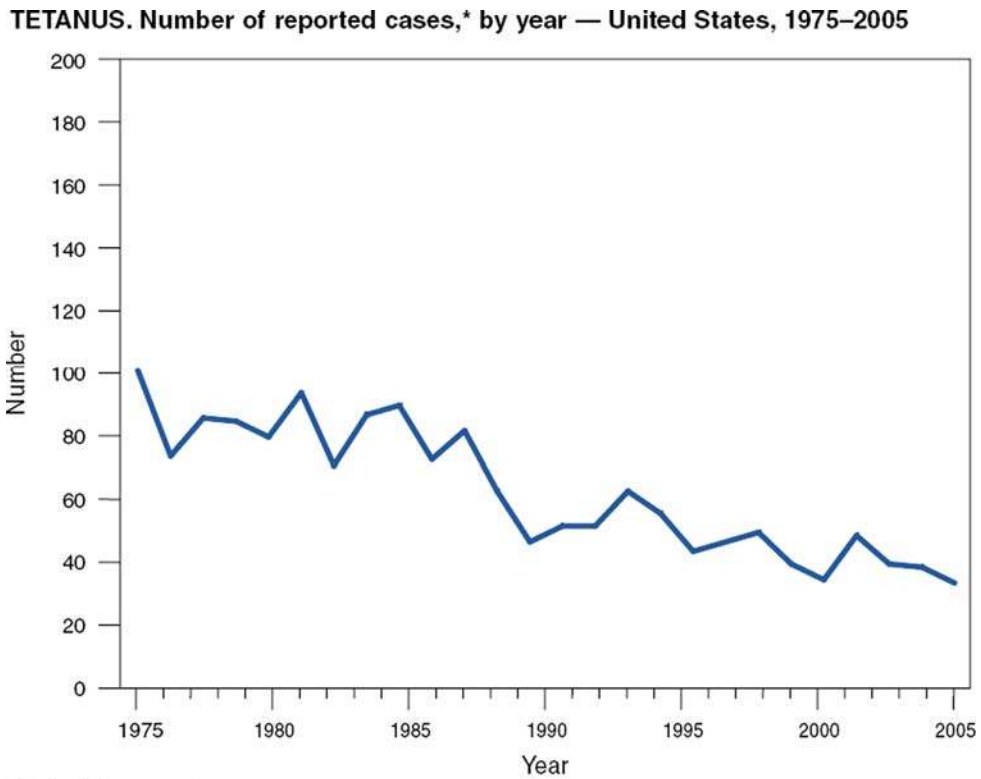


Figure 1. Tetanus: reported cases, United States, 1975–2008.

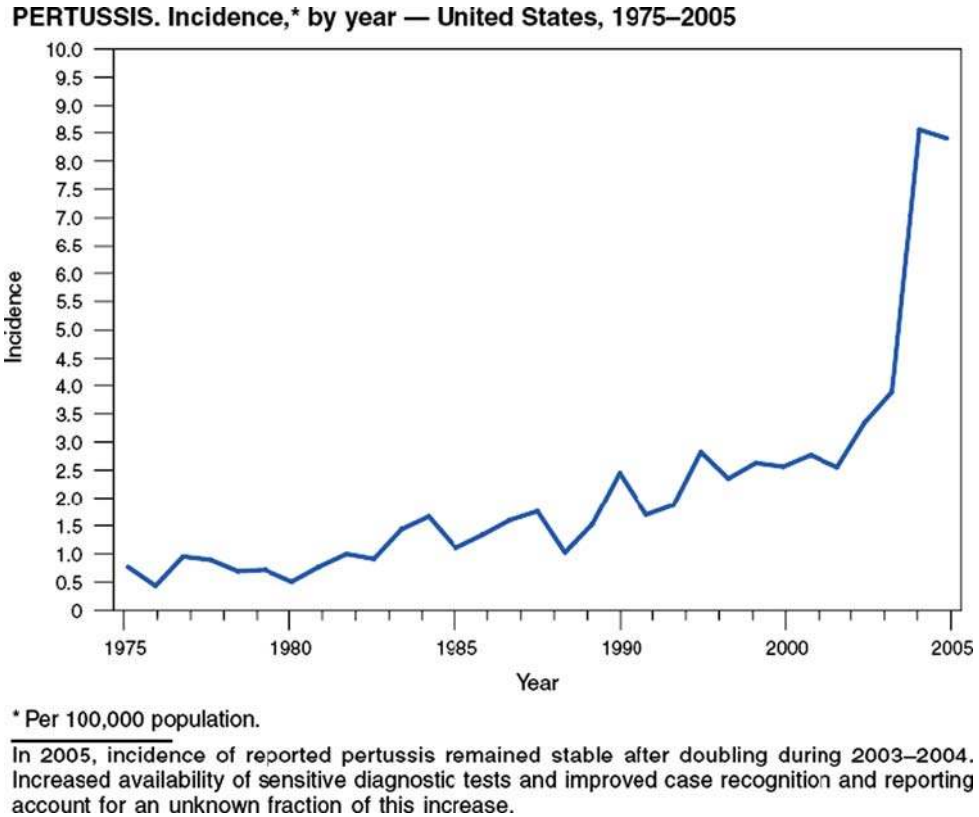


Figure 2. Pertussis: reported incidence rates per 100,000 population, United States, 1975–2008.

secular trend. For example, in considering pertussis, periodic increases in incidence approximately every 5 years can be seen on the background of the overall secular trend of decreasing incidence of the disease (Figure 2). The periodic trends represent variations in the level of immunity (herd immunity) to the etiologic agent influenced by natural infection, variation in the immunity levels of the population reflecting immunization, births, or migrating, or by changes in the antigenic composition of the agent.

The third trend is that of the *annual* variation, which frequently represents seasonal patterns. For example, foodborne diseases are associated with seasonal increases in the late summer and fall that may represent the influence of the ambient temperature on the ability of organisms to multiply in or on their reservoirs and sources, resulting in an increased concentration of organisms for potential contact with susceptible hosts. Additionally, the frequency of picnics and the lack of refrigeration add to the increased opportunity for small doses of agents to multiply to infectious doses.

The fourth time trend is that of the *epidemic* occurrence of the disease. If not noted earlier, an epidemic may be discovered by analyzing surveillance data. This has been seen when cases related to a common source are scattered over several health jurisdictions. For example, a *Salmonella*-contaminated food may result in the occurrence of cases over the distribution route of the food; the individual cases may not serve to alert any one health jurisdiction, but the collection of multiple cases may be distinct enough to be identified as an epidemic. Thus, surveillance can serve as an early warning system for epidemics.

When surveillance data are analyzed for time trends, it is necessary to compare these data with data collected over past years, to accurately interpret the current pattern of the occurrence of disease. Otherwise, changes in the occurrence of the disease may not be definable as being either normal or unusual variations.

8.3. Place

When analyzing surveillance data by place, it is important to recognize that the place represented by surveillance data may represent the area where the patient lives and not the area where the patient became infected. For epidemiological purposes, the important place(s) is where contact

occurred between the patient and the infectious agent or the place where the source of infection became infected. The place of interest will be determined by whether there is interest in control of the current occurrence of the disease or in prevention of future cases. Control measures directed at the site where the host came into contact with the agent can lead to control of additional similar cases immediately related to the initial case, but may not prevent additional cases if there are other sources of the organism that susceptible individuals may have contact with. For example, if a food is contaminated with *Salmonella* in the factory where it is prepared and the exposure of the host occurs in a restaurant serving that food, then closing the restaurant will not prevent cases from occurring in association with another restaurant that also obtained contaminated food from the same factory. Prevention of future cases can be accomplished by eliminating the source of contamination, in this instance, at the food-processing plant.

8.4. Person

Person factors to be defined in analyzing surveillance data may include age, sex, nationality, level of immunity, nutrition, lifestyle (such as sexual practices and intravenous drug use), socioeconomic status, genetic factors, travel history, hobbies, personal habits, and occupation. The evaluation of these factors, frequently referred to as risk factors, is important in further describing the occurrence of disease. For example, age-specific attack rates can be important in determining where control and prevention measures should be directed. Occupation may give a clue as to where intervention measures need to be directed. For example, in evaluating cases of brucellosis, knowing that abattoir personnel who work on the kill floors are at a higher risk of developing disease than personnel in other areas of the plant indicates where control measures need to be directed. In maintaining surveillance of avian influenza, it is important to determine if the patient had contact with avians or could it be the result of person to person transmission.

9. Reports

Appropriate reports should be prepared and distributed to those individuals who participate in the surveillance program as well as those who have a responsibility for preventive action. The purpose of the surveillance report is to communicate with people, to disseminate information, to educate the reader, and to direct, stimulate, and motivate the persons responsible for action. Reports can also be useful in acknowledging contributors to the surveillance activity. The report should not only summarize the surveillance data but also provide an interpretation of the analyses. Control and prevention measures can be discussed. Surveillance reports can also serve to alert the reader to impending problems, newer methods of control and prevention, current investigations, and new information developed from research or field investigations. They can also serve to stimulate better reporting by health-care practitioners.

Reports are usually prepared at regular intervals, such as weekly, monthly, quarterly, or annually. The frequency should reflect the interest in the data as well as the need for distribution of the data as related to control and prevention actions. During an epidemic, the immediate dissemination of data may be critical to stimulating reporting and to the institution of appropriate control measures; thus, daily or weekly reports may be indicated. It may be appropriate to distribute foodborne disease surveillance reports at weekly or monthly intervals during the summer and fall months due to the increased incidence of disease at these periods and then to reduce the frequency of reports during the remainder of the year. Special reports can be distributed as necessary; if rapid dissemination of the information is important, it can be distributed by express mail, telegram, telephone, fax, or computer. Rapid dissemination of information over a wide area may be of such critical importance that use of the public news media should be considered.

In the United States, almost all state health departments prepare and distribute at weekly or monthly intervals comprehensive surveillance reports that summarize their disease surveillance data. The national surveillance data are summarized by the CDC at varying intervals depending on the disease and its frequency. Those diseases reported weekly are summarized in the *MMWR*; other diseases are summarized in specialty surveillance reports that are distributed at regular intervals, from monthly to annually. An annual summary is prepared that summarizes in tables and figures all of the disease occurrence data reported by the states during the previous year and compares these data with data reported during the previous years. Characteristic of many of the CDC surveillance reports and especially of the MMWR is the interpretation of surveillance data and editorial comments that are integral parts of the reports. These reports are available to anyone who would like to receive them. The MMWR is now available by computer through the CDC's World Wide Web server at http://www.cdc.gov/mmwr. A hard copy of all of the CDC surveillance reports can be obtained from the Massachusetts Medical Society or the US Government Printing Office. World surveillance data are collected, summarized, and distributed by the WHO in a weekly and other reports.

10. Evaluation

Once a surveillance program has been developed and has been in operation for several years, it should be reviewed and evaluated. Even though the program is operational, it should not be assumed that it is effectively meeting the objectives of the surveillance activity. Thacker and Berkelman discuss evaluation[2] and the CDC has developed a guide for the evaluation of public health surveillance systems.[7]

11. Limitations of Surveillance

Surveillance of disease is dependent on a series of events that if not followed may prevent the case from being reported. The events include that the disease be severe enough that medical attention is sought and if sought, must be available; laboratory diagnostic facilities may be necessary and should be available; the health-care provider or his or her representative must report the case; and the respective health department must have adequate resources and direction to support the surveillance program. There needs to be consistency in the case definition, in the collection of data, and in the mechanism of reporting. Any alterations in these events may change the apparent pattern of disease. The consistency and stability of the occurrence of these events are vital to the development of reliable surveillance data. In addition to recognition of clinical cases, there may be many infected persons with mild or subclinical illnesses that are not noted in the routine surveillance system. For these cases to be noted, laboratory studies of the distribution of the organism or the antibody to it are required.

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CHAPTER 3

Molecular Epidemiology

Lee W. Riley

1. Introduction

Molecular epidemiology is now an established discipline in epidemiology.[1] It is the contemporary stage in the evolution of laboratory-based epidemiology that may have begun with the discovery in the late 1800s of ways to differentiate bacterial organisms by pure culture in artificial media.[2] Molecular epidemiology uses new molecular biology tools to address questions difficult or not possible to address by old laboratory tools. Just as statistical tools have become indispensable in epidemiological investigations and interpretations of epidemiologic data, molecular biology tools today have come to elucidate epidemiologic features of diseases that cannot be easily characterized by conventional techniques. Applied to infectious diseases, molecular biology methods have also come to challenge our traditional notions about the epidemiology of these diseases and have engendered novel opportunities for their prevention and control. This chapter will (1) review definitions commonly used in molecular epidemiology, (2) present an overview of molecular biology methods used to study infectious disease epidemiology, and (3) describe examples of the types of epidemiologic problems that can be addressed by molecular biology techniques, highlighting new concepts that emerged in the process of applying this approach to study bacterial infectious diseases.

2. Definitions

Molecular epidemiology: In Chapter 1, epidemiology is defined as "...the study of the distribution and determinants of health-related states, conditions, or events in specified populations and the application of the results of this study to the control of health problems". Correspondingly, molecular epidemiology can be defined as "the study of the distribution and determinants of health-related states, conditions, or events in specified populations that utilizes molecular biology methods". In molecular epidemiology of infectious diseases, the word "determinants" is emphasized. Furthermore, as we learn more about the biology of agents of infectious diseases, it is becoming evident that disease distribution in a community of hosts may be determined not only by factors such as the distribution of contaminated vehicles, host risk characteristics, and environmental contexts in which these diseases occur, but also by a pathogen's genetic composition.

For example, communicable bacterial pathogens that cause diarrhea are almost always transmitted by the fecal–oral route. Colonization of the intestine is an important prerequisite for these pathogens to facilitate their transmission to new hosts. Similarly, the agent of tuberculosis *Mycobacterium tuberculosis*, which is transmitted by the airborne route, must cause active disease in the host's lungs to assure its transmission to new hosts. An agent of leptospirosis, *Leptospira*, prolongs its survival in a community of hosts by establishing carriage in a mammalian host's kidney tubules so that it can be excreted in urine to infect other hosts. Mechanisms that underlie these organisms' ability to infect a specific target organ, which determines the specific pattern of transmission, are genetically determined. Thus, the definition of molecular epidemiology can be expanded to include the following: a study of the genetic factors that determine and regulate an organism's specific mode of transmission among hosts within an environmental context.

Differentiating molecular epidemiology from taxonomy and phylogeny: In February 2009, a bibliographic search by PubMed using the words "molecular epidemiology" generated 27,822 publications that contained these words in the title or the abstract. Most of these publications dealt with

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the laboratory methods used to subtype microorganisms. They did not discuss the use of these techniques to characterize disease occurrence, distribution, or determinants of disease distribution. Thus, the term "molecular epidemiology" is often used to describe studies that should be more appropriately called "molecular taxonomy" or "phylogeny". *Taxonomy* is the science of classification of organisms into naturally related groups based on a factor common to each. *Phylogeny* is the study of lines of descent or evolutionary development of an organism.[1]

Molecular epidemiology, taxonomy, and phylogeny may employ the same molecular biology laboratory techniques, but each is based on distinct principles. In taxonomy or phylogeny applied to infectious agents, the data that are generated are used to describe properties of organisms and their relationships to each other, based on an inferred model. Molecular epidemiology seeks to identify relationships between the etiologic agent of a disease (organism causing the disease), the host, and the environment in which the etiologic agent and the host reside. Both epidemiology and phylogeny as applied to infectious diseases may describe the distribution of particular genetic attributes of a pathogen over time. However, in phylogeny, the relationships of these genetic attributes over time must be guessed, since past evolutionary events cannot be empirically confirmed. That is, phylogenetic analysis seeks to infer past evolutionary events based on a model from a set of observations made with materials available in the present. Epidemiology focuses on events of the present or the recent past. It uses analytical and empirical study designs to identify factors that predict the relationship of attributes of pathogens to disease distribution in place, person, and time. It attempts to identify factors that determine disease transmission, manifestation, and progression. In molecular epidemiology, the hypotheses that are generated are testable or can be made to be testable empirically when enough study subjects are identified. Finally, epidemiology is always motivated by an opportunity or possibility for intervention and prevention. Thus, an application of a molecular biology technique to type an organism per se does not by itself constitute molecular epidemiology. However, it should be stressed that the disciplines of taxonomy and phylogeny play an important role in the discipline of molecular epidemiology, and this idea is reviewed in detail in a separate reference.[1]

Terminologies introduced in this chapter are based on definitions recommended by the European Study Group on Epidemiological Markers[3] and the Molecular Typing Working Group of the Society for Healthcare Epidemiology of America.[4] Others were adapted from a reference book *Molecular Epidemiology of Infectious Diseases: Principles and Practice*.[1] The terminologies below are mostly used in ways to apply to prokaryotic organisms.

Discriminatory power: The ability of a laboratory test to generate distinct and discrete units of information from different isolates, usually at a subspecies level.

Isolate: A population of microbial cells in pure culture derived from a single colony on an isolation plate and identified to the species level.

Strain: An isolate or a group of isolates exhibiting phenotypic and/or genotypic traits belonging to the same lineage, distinct from those of other isolates of the same species.

Clone: An isolate or a group of isolates descending from a common precursor strain by non-sexual reproduction exhibiting phenotypic or genotypic traits characterized by a strain typing method to belong to the same group.

Type, typing information, taxonomic unit, or marker: A specific and discrete unit of information or character belonging to a strain displayed upon application of a strain typing procedure. (e.g., antibiotic resistance pattern, serotype, electrophoretic banding pattern, allele).

Genotype: A genetic characteristic of a cell or an organism according to its entire genome or a specific set of genetic loci (allele).

Phenotype: An observable characteristic expressed by a cell or an organism, such as drug resistance, virulence, and morphology.

Biotype: A taxonomic unit of a microorganism belonging to one species based on a panel of biochemical tests.

Serotype: A taxonomic unit of a microorganism based on antigenic properties of a distinct set of surface structures.

Pathotype: A taxonomic unit of a microorganism that describes a pathogenic variant of a species or subspecies of microorganisms that normally colonize a host. Also called *pathovar*.

Allele: A nucleotide sequence variant of a gene at a particular locus.

Single nucleotide polymorphism (SNP): Small changes or variation in corresponding nucleotide sequences at a given locus (e.g., corresponding locus TTAGGTCCTTTA in strain 1 and TTTGGTCCTTTA in strain 2 differs by a single nucleotide in the third position).

Microsatellites: DNA sequences containing tandem repeats of a short sequence (e.g., CCG repeated 10 times in succession). Most often used in reference to such sequences in eukaryotic cells.

3. Methodology

It should be emphasized at the outset that molecular biology methods are no more special than any other methods—laboratory or non-laboratory—used in epidemiology. Curiously, however, when biochemical laboratory

methods are used to characterize organisms in an epidemiologic investigation, we do not use the term "biochemical epidemiology". For some reason, when molecular biology methods are used in an epidemiologic study, the study is described as "molecular epidemiology". This probably results from a peculiar human habit of glorifying certain characteristics over others. For instance, it is more fashionable in research universities to call a department doing the same type of research a "molecular biology" instead of a "microbiology" department. Molecular epidemiology is epidemiology. In truth, molecular biology methods are nothing more than one of many tools used in epidemiology to order data into separate units or strata so that they become amenable for different types of analytical procedures.

All laboratory-based methods used to type microorganisms (*typing systems*) fall into *phenotypic* and *genotypic* methods, as described by Maslow et al.,[5] Tenover et al.,[4] and Lipuma.[6] Most of the "conventional" laboratory typing methods fall into phenotypic methods, which are based on the detection of phenotypes or characteristics expressed by an organism. Strain typing methods based on genotypes rely on the analysis of nucleic acid contents and gene sequence polymorphisms (chromosomal DNA, extrachromosomal DNA, and RNA). Nowadays, with routine applications of molecular biology tests, the distinction between "conventional" and molecular biology laboratory techniques may no longer be meaningful. Many standard strain typing procedures today are built upon layers of tests that include both molecular and non-molecular biology techniques.

3.1. Phenotyping Methods

The first step in data stratification as applied to bacterial infectious disease agents is differentiating pathogens from normal commensal or saprophytic bacterial organisms. Commensal organisms reside, metabolize, and replicate in a healthy, immunocompetent host without causing disease. They colonize the skin, mouth, nasopharynx, gastrointestinal tract, vaginal mucosa, and others. A saprophyte is a type of microorganism that grows normally on dead matter, and hence is found in the environment. This initial step separates organisms into species. Microorganism characteristics used to define species include growth, morphologic, biochemical, serologic, functional, and physiologic properties.

3.1.1. Differentiation by Growth and Morphologic Characteristics. Differences in nutritional requirements of bacterial organisms determine their growth characteristics. They can be classified according to whether they grow at all or the way they grow on solid agar culture plates or in liquid media. Morphologically, they may be differentiated by their colony shape, color, texture, smell, or whether they "swarm" on the plate. They are also differentiated by the way they stain (e.g., Gram stain, acid fast stain) as observed under a light microscope, or their shape and size as observed under an electron microscope.

3.1.2. Typing Based on Biochemical Characteristics. Biochemical tests used to differentiate bacteria are based on the organism's metabolic activities. Strains differentiated by a panel of biochemical tests group into *biotypes*, and the tests are thus referred to as biotyping tests. The discriminatory power of a biotyping test depends on the number of biochemical tests used to subtype strains—the larger the number of biochemical characteristics analyzed in a pathogen, the greater the discriminatory power of the test. The tests depend on an organism's metabolic activities, which are sometimes subject to minor changes in growth condition (e.g., nutrient concentration, pH, and temperature) used, and hence the information that is generated may not always be reliable for epidemiologic studies. In developed countries, these tests have come to rely on kits and automated systems, facilitating rapid turnover. However, the large number of tests often needed to subtype a strain renders biochemical classification of pathogens expensive.

3.1.3. Typing by Serologic Characteristics. Another frequently used phenotyping method is based on a characteristic antigen-specific serologic (antibody) response induced by an invading organism in a mammalian host. Bacterial antigens that induce antibody response used for serologic classification include proteins and polysaccharides. For example, the species *Escherichia coli* characterized biochemically can be further subtyped into a "serogroup", based on the organism's characteristic somatic antigen (O-polysaccharide). A numerical designation is assigned to an *E. coli* strain carrying a characteristic O-polysaccharide that induces an antibody response (e.g., O157). The same strain, if further subtyped based on its flagellar protein (H-antigen), is classified as a "serotype". It will then be given an O:H designation (e.g., O157:H7). The genus *Salmonella* is biochemically separated into species and subspecies. They are further subtyped serologically according to their O and H antigens. Unlike *E. coli* serotypes, *Salmonella enterica* subsp. *enterica* (subspecies I) serotypes are given a descriptive name rather than a numerical designation, e.g., *S. enterica* serotype (or serovar) Typhimurium or *S. enterica* serotype Enteritidis. Nearly 1,500 serotypes of *S. enterica* subspecies I are currently recognized.[7] The specific serogrouping or serotyping assays include bacterial agglutination test with a panel of antigen-specific antisera, enzyme-linked immunosorbant assay (ELISA), latex particle agglutination, Western blot (or immunoblot) analysis, or a modification of these tests.

One limitation of serotyping is that different strains from the same species or even strains from different species

could carry cross-reacting antigens, which can result in false-positive tests. On the other hand, strains that do not express typing antigens are classified as non-typeable (e.g., non-typeable *Haemophilus influenzae*). Serologic tests, on the most part, are simple to perform, but they require maintaining a stock of typing polyclonal or monoclonal antisera. For example, a large number (~200) of antisera need to be used to serotype *Salmonella*. Thus, serotyping could be expensive and time consuming, and is usually confined to reference laboratories.

3.1.4. Typing by Functional or Physiologic Characteristics. Other ways to differentiate bacterial isolates involve examination of their response to specific manipulations. They include identifying differences in susceptibility to antimicrobial agents (antibiograms), lysis by or susceptibility to bacteriophages (phage typing), patterns of association with tissue culture cells (cell culture assays), toxigenicity, survival under in vitro or in vivo stress, and metabolic enzyme expressions (multilocus enzyme electrophoresis, or MLEE). These are reviewed in detail elsewhere.[1]

3.2. Genotyping Methods

Genotying or nucleic acid-based typing methods are ultimately based on the nucleic acid sequence differences in the chromosomal or extrachromosomal nucleic acid contents. As opposed to hundreds of ways to phenotype bacteria, all genotyping tests can be grouped into a variant of just three basic analytical procedures—(a) hybridization, (b) gel electrophoresis, and (c) nucleic acid sequencing. This allows for the use of common equipment and standard reagents to analyze many different types of infectious agents. Furthermore, genotypic characterizations of pathogens facilitate standard information storage and data analyses, interpretation, and communication, which are all amenable to computer-assisted manipulations.

In bacteria, genotyping analyses are based on comparison of two types of DNA: the genome and the extrachromosomal DNA. Typing systems based on the analysis of the genome fall into those designed to compare differences based on nucleotide changes that occur in parts of the genome (microdiversity) or the entire genome (macrodiversity). These typing methods, of course, had to be developed in place of direct whole genome DNA sequence analysis, which, despite recent advances in the sequencing technology, remains cumbersome and expensive for bacterial organisms (although no longer the case with viruses). Further detailed reviews on this topic are mentioned at the end of this chapter (Suggested Reading).

3.2.1. Extrachromosomal DNA Genotyping Methods. For bacteria plasmids are the extrachromosomal DNA elements used to type strains. Plasmids are covalently closed circular DNA that carry genes that may encode characteristics such as antibiotic resistance, virulence factors, or other functions that provide selective advantage to the organism but are not essential for its growth or survival. In fungi or protozoas, extrachromosomal elements used for typing may include mitochondria[8] and kinetoplasts (schizodeme analysis).[9]

Plasmid-based typing is an example of an electrophoresis-based strain typing procedure. It compares differences in molecular weight (MW) and the number of different plasmids a bacterium may carry. Nucleic acids and proteins are negatively charged. Plasmids, thus, can be resolved electrophoretically in an agarose gel matrix according to MW. That is, the separation of these molecules depends on their drag through the gel matrix as they migrate toward the positive charge pole. Large MW plasmids migrate more slowly in the gel matrix than low MW plasmids. Thus, depending on the number of different MW plasmids, each strain represented by a single electrophoretic lane will generate a banding pattern, a "fingerprint", or a profile, that can then be compared across multiple lanes (Figure 1).

In one early study designed to validate the discriminatory power of plasmid profile analysis of bacterial pathogens, Holmberg et al. compared *S*. Typhimurium isolates from 20 well-documented outbreaks by phage types, antimicrobial resistance patterns, and plasmid profiles.[10] They found that among nine outbreaks in which non-outbreak isolates were also examined, the plasmid profile analysis was able

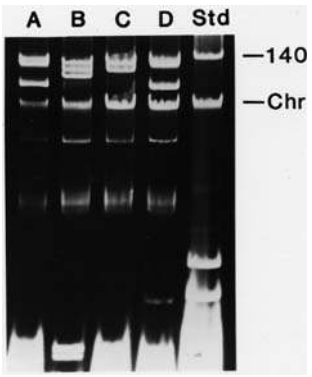


Figure 1. Agarose gel electrophoresis of plasmids (plasmid profile) extracted from drug-resistant *Salmonella enterica* serotype Typhimurium isolates from children with diarrhea in Brazil.[75] Lanes A–D show distinct banding patterns. All isolates carried a 140 MDa plasmid as well as lower MW plasmids. "Chr" denotes chromosomal DNA. MW marker standard is shown in lane marked "Std".

to distinguish outbreak-related isolates from non-outbreak-related isolates more frequently than the other methods (phage typing and antibiogram), suggesting that the plasmid profile analysis was more discriminatory than the other methods used to type strains at that time.

One drawback of the technique is that the procedure depends on the presence of at least one plasmid in a bacterium. If isolates carry only one or two plasmids, the patterns generated will not be sufficiently discriminating enough to distinguish the strains as related or unrelated. Isolates that contain only one or two plasmids of identical MW need to have the plasmids digested with endonucleases (enzymes that cut DNA molecules at a specific sequence site; see details under "Restriction Endonuclease Analysis or REA") to generate smaller fragments that can then be electrophoretically resolved. Another potential problem with this method is that plasmids on prolonged storage of a bacterium can be spontaneously lost, which can affect reproducibility of the typing method. Although plasmid profiles are relatively easy to perform and interpret, it does require a laboratory equipped for basic molecular biology techniques, including DNA extraction, restriction endonuclease digestion, electrophoresis, and photodocumentation of the results.

3.2.2. Genome-Based Typing Methods. In genome-based typing methods the entire genomic content of a microorganism is compared among different strains (macrodiversity analysis). There are three basic genome-based typing methods used in epidemiologic investigations, which are based on (1) allelic differences that occur within restriction endonuclease recognition sites in a genome, (2) comparison of multiple nucleotide sequences of whole genomes (called comparative genomics), and (3) whole genome hybridization patterns (see below for the description of hybridization) obtained from arrays of thousands of distinct short DNA fragments representing the whole genome fixed onto a glass slide or nitrocellulose filter ("gene chip" or microarray). The latter two methods have only recently begun to be applied to epidemiology.

3.2.3. Restriction Endonuclease Analysis (REA). Bacterial organisms express enzymes called restriction endonucleases that recognize unique sequences of several nucleotides in length and cut the DNA molecule at a specific position. They are called restriction enzymes because bacteria use these enzymes to eliminate (restrict) foreign DNA accidentally introduced into them. Thus one enzyme may generate distinct sets of DNA fragments from two different strains if the strains have a sequence difference at the corresponding restriction sites. Comparison of electrophoretic patterns based on the number and differences in MW of cut DNA fragments is called restriction endonuclease analysis (REA) or restriction fragment length polymorphism (RFLP) analysis.[11–13]

Enzymes that cut DNA molecules at recognition sites that occur frequently throughout a microorganism's chromosome are colloquially referred to as "frequent cutters". Electrophoresis of DNA fragments generated from such enzymes will produce thousands of overlapping bands upon gel electrophoresis, and the resulting patterns become difficult to interpret (Figure 2A). Two different ways have been developed to overcome this limitation, as outlined below.

3.2.4. Southern Blot Hybridization. Southern blot hybridization analysis, named after its developer,[13] is a type of analysis that facilitates detection of a particular DNA fragment of interest among hundreds of other DNA fragments generated by REA. DNA fragments separated in an agarose gel are first transferred onto a piece of nitrocellulose or nylon membrane. The membrane is then exposed to a piece of

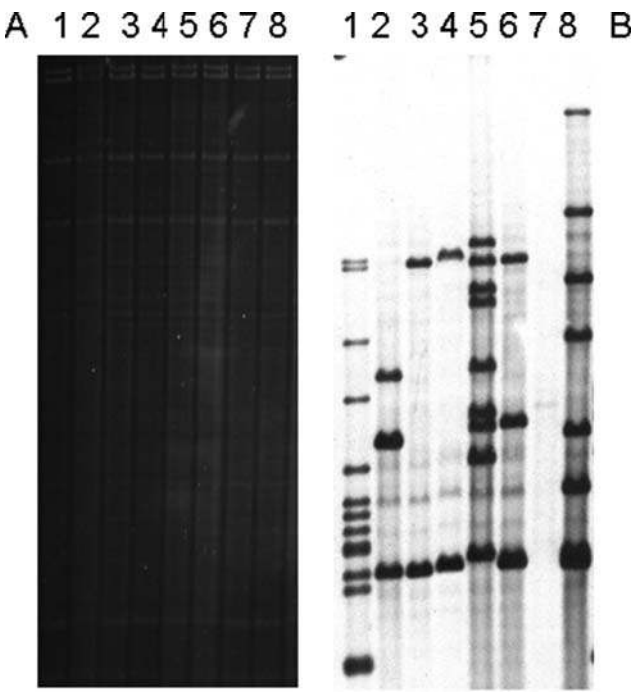


Figure 2. Restriction fragment length polymorphism (RFLP) analysis with Southern blot hybridization of eight clinical isolates of *M. tuberculosis*. Panel (**A**) shows *M. tuberculosis* DNA digested with a restriction endonuclease PvuII. Note the smear pattern in each lane, representing thousands of DNA fragments resolved by electrophoresis in an agarose gel. However, when the DNA fragments of the gel are transferred onto a piece of nylon membrane and hybridized with a probe directed at *M. tuberculosis*'s *IS6110* insertion element, only the DNA fragments that contain this element becomes visualized (**B**). Thus, the patterns generated in the membrane in panel B become interpretable and can be compared among strains.

single-stranded DNA (probe) labeled with a molecule that facilitates visual detection of a selected target DNA fragment. The probe will specifically bind (hybridize) to its complementary DNA sequence embedded in the membrane. For example, a nucleic acid sequence or probe *ATCGGCGATG* will hybridize to a target containing the sequence *TAGCCGCTAC*. Thus, this probe-based detection of target DNA fragments is called hybridization. If the organism of interest harbors more than one copy of the target DNA sequence in different locations in the chromosome, the membrane will show multiple bands (banding pattern) in a single lane, which can be visually detected. Of course, the number of such visualizable bands will be few and will not be obscured by the other fragments in the membrane, which cannot be visualized because they do not hybridize to the probe. This allows for comparison and interpretation of the band patterns (Figure 2B).

This method has been applied to subtype a wide variety of bacterial organisms, and is the current standard method used to type *M. tuberculosis*, in which the organism's repetitive DNA element or insertion element called IS*6110* is used as the target for a hybridization probe (called *IS6110* RFLP).[14] One limitation of this approach is that the DNA sequence selected as a target for a hybridization probe must be present in the chromosome in high copy numbers (usually >5) for the technique to be sufficiently discriminating. Clinical isolates of *M. tuberculosis* completely lacking or having small copy numbers of IS*6110* have been found.[14] In such situations, secondary typing methods must be applied. Another drawback of the RFLP analysis followed by hybridization is that it requires a specialized reference or research laboratory to perform.

When the hybridization typing method uses ribosomal genes (*rrn*) as a target, it is called ribotyping. Ribosomal RNA sequences are highly conserved, especially within members of the same genus. This property can be used, then, to type a wide range of organisms related at the species level, but by the same token, the discriminatory power of ribotyping is low. One study found that *E. coli* isolates obtained from widely separate geographic areas shared identical ribotypes.[15] Thus, for *E. coli*, ribotyping would not be discriminating enough for local epidemiologic studies or regional surveillance, although it may be useful for studying evolutionary relationships.

3.2.5. Pulsed Field Gel Electrophoresis (PFGE). The second way developed to overcome the problem of uninterpretable electrophoretic patterns generated by RFLP is to use endonucleases that recognize rare restriction site sequences ("rare cutters"). Such enzymes will produce very large DNA fragments, which, when resolved electrophoretically, will generate interpretable patterns. However, electrophoresis in agarose gels of large pieces of DNA introduces two additional technical constraints. Linear pieces of DNA $\geq$25,000 bases (25 kilobases or kb) are difficult to resolve by the conventional agarose gel electrophoresis method. They get stuck during migration across the gel matrix. Thus, PFGE was developed in which the orientation of the electrical field across the gel is changed (pulsed) periodically.[16] This allows the DNA fragments to move in different directions, releasing them from the stuck position. The DNA fragment will thus take a path of least resistance along its gel migration toward the positive charge pole.

The second technical obstacle that had to be overcome was the tendency for large DNA fragments to undergo spontaneous autodigestion. This problem was eventually solved by enzymatically lysing and digesting the cell wall and proteins of the test bacterial organism embedded in an agarose gel plug. This releases the bacterial DNA directly into the plug. The released DNA can then be digested within the plug with a restriction enzyme. This prevents autodigestion.

The major advantage of PFGE is that virtually all genome-containing organisms are typeable by this method. In general, genetic changes detected by PFGE reflect genetic events in an organism that occurred in the recent past. Hence, it is a highly informative technique with wide epidemiologic appeal. PFGE is the method of choice used by a national foodborne disease surveillance system called PulseNet, which is collaboration among Centers for Disease Control and Prevention (CDC), Association of Public Health Laboratories (APHL), and the food regulatory agencies, including the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA). Foodborne pathogens included in the PulseNet surveillance currently include *Salmonella* Typhimurium, *E. coli* O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Shigella sonnei*[17] (www.cdc.gov/pulsenet). PulseNet has contributed to detecting, investigating, and controlling multistate outbreaks of *E. coli* O157:H7,[18] *Salmonella* Agona infections,[19] and listeriosis.[20] The network will expand to PulseNet Canada, PulseNet Europe, PulseNet Asia Pacific, and PulseNet South America.

One major limitation of PFGE is that it is technically demanding, requiring an expensive electrophoresis apparatus. Thus, this typing system is currently used only by reference or research laboratories.

3.2.6. Whole-Genome Sequence Comparison. The reduced cost and ability to rapidly sequence large pieces of DNA engendered a new discipline called comparative genomics.[21, 22] With viruses, whose genomes are considerably smaller than those of bacteria, comparative genomics is already applied to directly genotype clinical isolates. That is, hundreds of genomes of viral strains belonging to one species

can now be compared rapidly to each other and applied to address epidemiologic questions. For example, it was the comparative genomics analyses that enabled investigators to conclude that the agent of severe acute respiratory syndrome (SARS) belonged to a group of coronaviruses.[23]

With bacteria, because of the large genome, such comparisons are still not possible for a large number of strains. That is, hundreds of genomes of a single bacterial species cannot be compared because they have not been sequenced. However, new information obtained from comparison of a limited number of whole genome sequence databases is used to develop alternative genotyping methods. For example, at least eight different clinical isolates of *Staphylococcus aureus* have now undergone whole genome sequence analysis. Multiple alignment comparisons reveal several regions of difference. One such region called RD13 containing a cluster of Staphylococcus exotoxin (*set*) genes has been recently exploited to develop a new genotyping method called exotoxin sequence typing.[24]

Whole genome sequence comparison of *M. tuberculosis* H37Rv, CDC1551, strain 210, and *Mycobacterium bovis* AF2122/97 led to the identification of 212 *single nucleotide polymorphism* (SNP) markers.[25] More recently, an analysis of these SNP markers in a global collection of 294 *M. tuberculosis* strains identified six phylogenetically distinct SNP cluster groups (SCGs). SCGs were strongly associated with geographic origin of these isolates.[26]

3.2.7. Microarray Comparisons. Another way to compare whole genomes is based on hybridization patterns generated from thousands of short pieces of DNA (probes) of known sequence representing the genome, arranged on a glass slide or nitrocellulose membrane, often referred to as microarray or "gene chip". Microarrays are a type of DNA hybridization matrix typing method described previously. The hybridization technology is not new, and the microarray technology is just a recent variant of the technology made possible by miniaturization techniques developed in the semiconductor industry.[27–29]

Most microarray-based applications to infectious disease currently focus on taxonomic differentiation or diagnosis, and not on epidemiologic investigations. In one study, a whole genome DNA microarray analysis of 13 vaccine strains of *M. bovis*-BCG found 16 regions deleted in BCG strains relative to the sequenced *M. tuberculosis* H37Rv genome.[30] Kato-Maeda et al. found 25 deleted regions among 19 clinical *M. tuberculosis* isolates by a whole genome microarray approach.[31] Here, they reported that there was an inverse relationship between the proportion of deleted regions in these strains and the percentage of patients with cavitary disease infected with these strains. The discovery that *M. tuberculosis* contained such regions of deletion led some investigators to propose a genotyping scheme called "deligotyping".[32] In this method, clinical isolates of *M. tuberculosis* are compared according to differences in the regions of deletions.

Microarrays facilitate comparison of a large number of strains more rapidly than the whole genome sequence comparisons, but they are dependent on the number and types of DNA fragments that are included in the array, and hence will not be as discriminatory as the latter. Furthermore, they require specialized laboratories equipped with a scanner to read the microarray and expensive software to interpret the hybridization patterns on the chip. Thus, this technology is not likely to become readily available in the near future in most places to conduct epidemiologic investigations.

3.2.8. PCR-Based Genotyping Methods. All of the genotyping methods described above are based on DNA extracted from bacteria that are cultivated in vitro. Culturing bacteria serves two important purposes—(1) makes available large amounts of DNA for the genotype analysis and (2) assures that the genetic material being analyzed represents a distinct bacterial strain, obtained from pure culture.

For bacterial organisms that grow very slowly (*M. tuberculosis*) or cannot be cultivated artificially (*Treponema pallidum*), the above genotyping methods would be difficult to apply. Improper storage of bacterial isolates may also cause them to die, in which case genotyping by any of the above methods will not be possible. Polymerase chain reaction (PCR)-based tests can overcome these limitations. The PCR technology is a powerful and simple molecular biology tool, which is now applied in a variety of ways to genotype microorganisms in taxonomic as well as epidemiologic studies. It should be noted that PCR is only one of several methods called nucleic acid amplification tests (NAAT) used to make multiple copies of a target DNA or RNA of an organism or cells in vitro without culturing the organism or cells. PCR is perhaps the most frequently used NAAT and the most common method applied to subtype microorganisms. There are many excellent books that detail the procedure and are listed at the end of this chapter (Suggested Reading).

3.2.9. Overview of PCR. There are a number of frequently used terms that the reader should become familiar with in the discussion of PCR tests. Those familiar with the procedure may skip this section.

PCR involves multiple cycles of a three-step process: (1) denaturation of double-stranded DNA into single strands, (2) annealing of oligonucleotide primers to target DNA sequences called a template, and (3) primer extension along the template sequence from the primer binding site. These steps are carried out in a plastic microcentrifuge tube containing a reaction mixture of buffers, nucleotides, primers, heat-stable polymerase, and a sample DNA template. DNA

when heated to a sufficiently high temperature will separate into single strands. Separation into single strands of a double-stranded DNA is called denaturation. Thus, in the first step, the sample DNA (template) is usually heated to 95–100°C. Primers are synthetic single strands of DNA (oligonucleotides) of variable lengths designed to bind (anneal) to specific complementary target sites in a DNA template. They can be custom-made commercially. Primers bind to the target sequences when the temperature of the reaction mixture is decreased from a denaturation temperature to an annealing temperature range (~40–60°C). At about 70–75°C (primer extension temperature), a thermostable enzyme called DNA polymerase will add nucleotides (extension) complementary to the unpaired DNA strand onto the annealed primer. In this way, a DNA segment located between the two annealed primers will be copied. This three-step cycle involves change in temperature only—denaturation, annealing, and extension temperature—which is facilitated automatically by a programmable heating device called thermocycler. Depending on the time applied to each of the steps in the cycle, the type of thermocycler used, and quality of the polymerase, a single copy of DNA can be multiplied to more than a billion copies after 30 cycles in less than 3 h. The amplified product can then be visualized in an electrophoretic gel under UV illumination after staining with ethidium bromide.

For PCR tests, the cultivation of organisms is usually needed not so much to make enough nucleic acid material available for analyses, but to assure that the product being analyzed represents a single or pure strain of an organism. For bacteria, if a clinical specimen is obtained from a normally sterile body site such as blood, cerebrospinal fluid, or deep tissue, and it is obtained in a sterile fashion, PCR assay may be performed directly on the specimen itself. The primer sequences could be designed to bind to sequences that are specific to a target organism (e.g., IS*6110* of *M. tuberculosis*). In this way, DNA targets in an organism infecting nonsterile sites (sputum, urine, stool) could be amplified. Blood, urine, and other tissues may contain products inhibitory to PCR, but if the specimen contains a sufficiently large number of the infectious agent of interest, the desired target DNA could be amplified and detected. If a stored organism becomes contaminated with other organisms, or lose their viability, a PCR test can still amplify DNA from that organism, as long as the DNA material is intact. If the primers are highly species specific, a PCR test can distinguish the target sequences from DNA derived from contaminating organisms, thus precluding culturing the target organism from a specimen.

3.2.10. PCR-Based Strain Typing Methods. PCR-based genotyping methods that compare electrophoretic bands are classified into two general categories: (1) those based on MW polymorphism of a single amplified product and (2) those that display band patterns (fingerprints) from multiple amplified products. The former method is often used to type organisms to predict species,[33–35] serogroups, serotypes,[34, 36, 37] or *pathotypes*.[37] The major advantage is that this PCR-based method is considerably simpler than the conventional methods used to group them into species, serotypes, or pathotypes, but its discriminatory power is limited to differentiating organisms belonging to a large taxonomic unit. It does not discriminate beyond these taxonomic units.

More discriminating PCR-based typing methods require generation of fingerprints. These latter techniques can be divided into three major groups (Table 1): (1) those that rely on sequence polymorphism in the whole genome,

Table 1. PCR-Based Strain Typing Methods Used in Epidemiologic Studies

General category of methods	Specific techniques
Methods that rely on sequence polymorphism in the whole genome	Arbitrarily primed PCR (AP-PCR) or randomly amplified polymorphic DNA (RAPD) PCR
Methods based on heterogeneity within endonuclease restriction sites	PCR-restriction enzyme analysis (REA) or PCR-restriction fragment length polymorphism (RFLP) analysis
	Amplified fragment length polymorphism (AFLP) analysis
	Restriction-site-specific PCR (RSS-PCR) analysis
	Amplified ribosomal DNA restriction analysis (ARDRA)
Methods based on interspersed DNA repetitive elements	Extragenic palindromic (REP) assay
	Enterobacterial repetitive intergenic consensus (ERIC) sequence assay
	Mosaic repetitive element PCR (BOX-PCR)
	Insertion element PCR assays (IS6110, IS1245, IS900)
	Short interspersed DNA repetitive element assay (PGRS, VNTR, MPTR, ETR)
	Direct repeat (DR) PCR or spoligotyping
Methods that compare allelic differences in selected target genes by sequencing PCR products	Multilocus sequence typing (MLST), single nucleotide polymorphism (SNP) analysis

(2) those based on heterogeneity within known restriction endonuclease recognition sites, and (3) those that take advantage of repetitive elements interspersed in the target genome.

One relatively new category of PCR-based typing methods is based on comparison of sequences of PCR products of selected gene targets instead of comparison of band patterns by electrophoresis. One example of such a method is called multilocus sequence typing (MLST), discussed below. All of the above methods are described in detail in reference (1). Methods that are most frequently used in epidemiologic investigations are described below.

3.2.11. PCR Typing Methods Based on Sequence Polymorphism in the Whole Genome. Arbitrarily primed PCR (AP-PCR), also called randomly amplified polymorphic DNA (RAPD), are PCR techniques that rely on arbitrarily or randomly designed sequences of primers that anneal specifically to DNA templates in a target organism.[38–40] If a set of these randomly designed primers anneal to target DNA sequences, the intervening segments that are proximal enough to these annealing sites can be amplified and generate products of different MW. Such products will display a band pattern in an electrophoresis gel for each strain. The advantage of this method is that the target nucleotide sequence does not have to be known. The primer sequences are determined empirically until a set that works is found. The final choice of the primer design is based on the generation of reproducible and interpretable band patterns. The major drawback of this test, however, is its reproducibility. Because this technique is based on random sequences of the primers, there is always a risk that contaminating DNA may get amplified. To minimize these nonspecific amplification, the following precautions are taken: (1) DNA templates should be from organisms recovered in pure culture, (2) include a sample in which no template is added (negative control), (3) standardize template DNA concentration in all samples to be tested, and (4) if possible, test all the desired samples in one batch.

3.2.12. PCR-Restriction Enzyme Analysis. The same principles behind REA, RFLP, and PFGE analyses of genomes of pathogens can be applied to PCR products. A PCR product, if it is sufficiently large, 1,000–2,000 base pairs or bp, can be digested with an endonuclease, and the generated DNA fragments can be resolved by gel electrophoresis and visualized. If such amplified products contain nucleotide changes at the restriction sites, varied electrophoretic band patterns will result from the digested PCR products. One drawback of this PCR-REA (or PCR-RFLP analysis) method is that part of the target sequence to be amplified has to be known ahead of time.

3.2.13. PCR Typing Methods Based on DNA Repetitive Elements. Repetitive DNA sequence elements are found in both prokaryotic and eukaryotic chromosomes. A variety of repetitive DNA elements have been identified in bacterial pathogens, which have been exploited to develop PCR-based strain typing assays. All of these procedures use the same approach, in which primers are designed to anneal to target repetitive sequences in the outward direction (outward primers) near the ends of these repetitive elements. The newly synthesized DNA strand extends away into the nonrepetitive sequences. Thus, sequences located between the elements are amplified. Because these elements are interspersed in the chromosome at different locations between species or strains, the amplified products will be of variable number and MW. The size of the amplified products will depend on the distance between the repetitive elements and the quality of the polymerase, and other PCR conditions used. Of course, longer the distance between the repetitive elements, harder it is to amplify such a region.

Table 1 lists some of the major bacterial repetitive DNA elements that have been used for strain typing. One frequently used repetitive element genotyping method is called enterobacterial repetitive intergenic consensus (ERIC) sequence PCR.[41, 42] ERIC sequences are dispersed throughout the bacterial chromosome, especially of Gram-negative bacteria, and are located in extragenic regions.[41, 43] The exact function of ERIC sequences is not known.

Another repetitive element used for typing by PCR is called BOX, first reported in Gram-positive bacterial organisms.[44] BOX elements include mosaic combinations of three subunits, boxA (59 bp), boxB (45 bp), and boxC (50 bp),[44] which are all found in *Streptococcus pneumoniae*. Gram-negative organisms such as *E. coli* and *S. typhimurium* contain *boxA*-like sequences, but not *boxB* or *boxC* sequences. BOX elements have been widely applied to characterize the epidemiology of pneumococcal diseases.[45–48]

3.2.14. Insertion Sequence (IS)-Based PCR. One potential disadvantage of PCR tests based on cross-species, widely distributed repetitive elements like ERIC and BOX, is the possibility for a nonspecific amplification when DNA from one organism is contaminated with DNA from another. In some bacterial species, this potential problem can be overcome by techniques that target repetitive elements that are unique to the species.

Bacterial organisms carry certain genetic elements that "jump", translocate, or transpose to new locations in the chromosome. Transposable elements that carry no genetic information except that needed by the elements to insert themselves into a new site are called simple transposons or insertion sequences (IS). Some insertion sequences are

found across species while others are species specific. If the copy number of an IS element in a bacterial strain is high enough and if it is randomly distributed in the chromosome, DNA sequences between these elements can be amplified, as described in repetitive element PCR assays above.

One example of a widely targeted IS element used to type a bacterial organism is IS*6110* of *M. tuberculosis*. IS*6110* is an IS unique to the members of the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis, M. microti*, and *M. africanum*). IS*6110* has been used as a target as well as a probe for RFLP-Southern blot fingerprint analysis of *M. tuberculosis* strains. Ross and Dwyer reported a PCR-based method designed to amplify sequences between IS*6110* elements to generate DNA fingerprint patterns.[49]

Of course, for IS*6110*-PCR-based typing method to work, the IS element has to be present in large numbers in *M. tuberculosis*. Thus, methods that take advantage of other different interspersed short repetitive DNA elements present in *M. tuberculosis* have been developed. There are at least four such short repetitive elements used to perform PCR-based genotyping of *M. tuberculosis*—(1) the polymorphic GC-rich tandem repeat sequence (PGRS), (2) the major polymorphic tandem repeat (MPTR) loci, (3) the exact tandem repeat (ETR) loci, and (4) the direct repeat (DR) elements located at a single locus in *M. tuberculosis* complex strains (Table 1).

PGRS elements together with IS*6110* were exploited for a PCR-based typing of *M. tuberculosis* strains by a method called double-repetitive-element (DRE) PCR.[50] The greater number of repetitive elements represented by PGRS and IS*6110* increases the chance of amplifying sequences between these elements. When the DRE-PCR method was compared to the standardized IS*6110* RFLP analysis of *M. tuberculosis* strains during a TB outbreak in a prison in Havana, Cuba, the two methods identified the same clusters of TB patients who were clearly linked epidemiologically in the prison, which validated the PCR-based test for epidemiologic application.[51]

Like most PCR-based genotyping tests, the DRE-PCR test suffers from low reproducibility. However, because of its simplicity, the DRE-PCR assay may be useful for screening a large number of isolates prior to using a more labor-intensive but discriminating test such as IS*6110*-RFLP.

The variable numbers of tandem repeats (VNTR) found in the MPTR and ETR loci in *M. tuberculosis* have also been exploited to type this pathogen.[52–54] In *M. tuberculosis*,the MPTR contains 15-bp repeats with a single consensus sequence and high sequence variability between these repeats.[55, 56] Each ETR locus contains large tandem repeats with identical repeat DNA sequences and each locus has a unique repeat sequence.[53] Thus, the number of these repeat units at each MPTR or ETR locus can be predicted from the size of the PCR products, which becomes the basis for VNTR genotyping. VNTR genotyping has been applied to a variety of bacterial organisms.[57–59] A recent variant of this method applied to type *M. tuberculosis* is called mycobacterial interspersed repetitive unit (MIRU) VNTR, which is based on *microsatellite*-like sequences scattered throughout the *M. tuberculosis* chromosome.[60, 61] By using 12 of these MIRU loci, several investigators have demonstrated that the resolution achieved by this typing method is comparable to that of IS*6110*-RFLP typing method.[62–64]

Another PCR-based method used to type *M. tuberculosis* is based on a region called direct repeat (DR) locus. This locus contains multiple copies of a repeat 36 bp sequence interspersed with unique spacer sequences which are 34–41 bp long.[55, 65] A typing method based on this variation in the spacer sequences is called spoligotyping (spacer oligotyping).[66] Oligonucleotides representing the spacers are synthetically made and covalently attached to a piece of membrane. This membrane then is used as a target for hybridization against PCR products obtained from amplification of the spacer sequences located between the DRs. PCR products used as probes are generated from the total genome of a strain of *M. tuberculosis* by one set of primers designed to amplify spaces between the DRs in the test strain. If the membrane contains an oligonucleotide representing a spacer sequence also present in the test strain, an amplified product will hybridize to it. This will generate a hybridization matrix, not unlike the microarray discussed earlier. Thus, unlike the previously described PCR-based typing methods, the patterns that are generated and compared are not electrophoretic fingerprints.

One important advantage of spoligotyping over the standardized IS*6110*-based method[14] is that it reliably differentiates *M. tuberculosis* from *M. bovis*, two members of the *M. tuberculosis* complex.[66] This is because of the low copy number of IS*6110* in *M. bovis*. Discrimination of clinical isolates of *M. tuberculosis* by spoligotyping followed by DRE-PCR performed in French West Indies has been found to be almost as discriminating as the reference IS*6110*-RFLP method.[67] The spoligotyping membrane is commercially available. Thus, any laboratory capable of performing PCR should be able to characterize *M. tuberculosis* isolates by spoligotyping.

3.2.15. Multilocus Sequence Typing (MLST). Many other PCR-based genotyping methods for bacterial pathogens have been described. They all use variations of the general theme described above (Table 1). One relatively new method called multilocus sequence typing (MLST) combines PCR assay with sequencing to assign a sequence type designation to a strain. Standardized MLST analysis

for most bacterial organisms is based on sequencing a PCR-amplified region from each of a set of unlinked housekeeping genes. For example, with *S. aureus*, these genes include seven genes: *arcC, aroE, glpF, gmk, pta, tpi*, and *yqiL.*[68,69] Each of the amplified segments from these genes shows potential allelic variation in different *S. aureus* isolates. An MLST clone that is designated as a particular sequence type (ST) is defined as a strain belonging to a set of isolates with identical sequences at each of the seven loci. A methicillin-resistant *S. aureus* (MRSA) strain designated ST8 (also called PFGE type USA300) has recently been reported to cause widespread community outbreaks of severe skin and deep tissue infections in different regions of the United States.[70, 71] Because this is a sequence-based method, it is highly reproducible across different laboratories, and thus standardized protocols have been developed for several bacterial organisms. Protocols as well as ST designations for these organisms are maintained and accessible at http://www.mlst.net.

4. Epidemiologic Problems Addressed by Genotyping Techniques

At least six common epidemiologic concerns related to bacterial infectious diseases are addressed by genotyping tests. They are (1) characterizing distribution and dynamics of disease transmission in geographically widespread areas; (2) identifying and quantifying risk in sporadic occurrence of infectious diseases; (3) stratifying data to refine epidemiologic study designs; (4) distinguishing pathovars from nonpathovars; (5) addressing hospital and institutional infectious disease problems; and (6) identifying genetic determinants of disease transmission. These are discussed separately below.

4.1. Studying Dynamics of Disease Transmission in Geographically Widespread Areas

Much of the literature that applies molecular biology tools to genotype bacterial pathogens addresses this epidemiologic issue—characterizing geographic distribution of clones or clonal groups. Of course, this is an important component of epidemiology, but unfortunately these types of studies are somewhat limited in their ability to provide good understanding of the transmission dynamics of an infectious disease that yields practical solutions to disease prevention and control. Very often, these studies use convenient samples, in which available collection of microorganisms is genotyped first and questions are asked later. A typical question that is asked afterward is "What do we do with the strain type data?" When no other types of data are collected (i.e., demographic characteristics of the human subjects from which the isolates are obtained, exposure measures, clinical features, etc.) the analysis of the isolates is limited to just describing the number of clones, the proportion that each clone contributes to the study collection, speculations about which clone descended from which, and their geographic distribution. Unfortunately, these types of data contribute little to public health.

For molecular epidemiology to be truly relevant, the right questions need to be asked first before any strains are genotyped. Tracking a strain from place to place and over time is one application of molecular biology techniques, but such an application is not sufficient to address important and relevant questions about disease transmission. In infectious disease epidemiology, researchers and practitioners ask the following types of questions[1]: How does an organism get introduced into a community, and how and why does it spread? What are the risk factors for infection and transmission in a community and how is the infection maintained in a community of hosts? How and why does a particular strain suddenly emerge to become the predominant strain in a community, and why does it disappear? How is drug-resistant infectious disease prevalence in a community affected by fluctuations in clonal distribution over time in that community? How is the prevalence of spectrum of clinical manifestations (mortality, severe disease, asymptomatic infection) of an infectious disease affected by clonal distribution of a pathogen in a community? Do unique biologic characteristics of a member of a bacterial species contribute to enhanced transmission and hence increased prevalence of that member in a community or institution? How do we use the knowledge we gain about the dynamics of disease transmission to implement effective disease control programs? How do we measure the effect and impact of the intervention made based on the knowledge acquired from the use of a particular laboratory test? Once these questions are asked, one can then decide how to collect the isolates, which collection of isolates should be genotyped, what comparison isolates (controls) should be collected, and which of the many strain typing techniques should be used.

4.2. Identifying and Quantitating Risk in Sporadic Occurrence of Infectious Diseases

When a disease occurs as part of a recognized outbreak, it is possible to identify vehicles or risk factors for that illness. This is because in an outbreak, an analytical study

(case–control, cross-sectional, or cohort) can be designed to implicate a source or reservoir of an infectious agent responsible for the outbreak. If a risk factor or a vehicle of an outbreak is identified, the number of cases in that outbreak attributable to that risk factor (attributable risk fraction) can be calculated. In an outbreak, this would be 100% minus the background disease prevalence for that place. Unfortunately, in places like the United States, it is estimated from surveillance reports that less than 20% of reported cases of foodborne diseases, such as salmonellosis, come from recognized outbreaks.[72] The others are reported from sporadic cases. It is extremely difficult, if not impossible, to identify risk or estimate attributable risk fraction for sporadic diseases. The ability to genotype bacterial isolates from sporadic disease cases provides an opportunity to identify risks and calculate attributable fractions for those risks. One early example of such a study follows.

In late summer of 1981, case–control studies of two different salmonellosis outbreaks in New Jersey and Pennsylvania caused by *S*. Newport and *S*. Typhimurium implicated precooked roast beef produced at a meat processing plant in Philadelphia as the vehicle.[73] The distribution of the contaminated beef, of course, was not limited to just these two outbreak settings. It was distributed statewide in both New Jersey and Pennsylvania. The question that was asked was, "what proportion of the salmonellosis cases in these two states during this period that were not part of the two recognized outbreaks were caused by consumption of the contaminated precooked roast beef?" That is, the question asked for the proportion of all sporadic cases of salmonellosis in the two states attributable to the contaminated precooked roast beef.

None of the *S. typhimurium* isolates contained any plasmids, so this technique could not be applied to further subtype this *Salmonella* serotype. *S. newport* isolates from the two outbreaks and an isolate from the implicated meat product, however, contained identical 3.7 and 3.4 MDa plasmids.[74] *S. newport* isolates from persons in the two states not involved in the outbreaks were obtained from the respective state health department laboratories from the summer of 1981. In addition, *S*. Newport isolates from the same states before and after this summer were from other states during the same time period (geographic control), was obtained and analyzed. Telephone interviews of the people who got sick during the summer of 1981 revealed that a significantly greater proportion of those infected with a strain containing the 3.7 and 3.4 MDa plasmids ate precooked roast beef shortly before they developed diarrhea.[74] In the summer of 1981, nearly 45% of the *S*. Newport isolates from sporadic cases of salmonellosis in New Jersey and Pennsylvania belonged to this strain. Thus, the fraction of all sporadic *S. Newport* diarrhea cases during this period that could be attributed to this single contaminated product was estimated to be 45%.

This study demonstrated that the incidence of diseases-like salmonellosis in a widespread geographic region could be rapidly and greatly affected by the introduction of a single contaminated food product. In fact, even after recall of the contaminated product, *S*. Newport strains associated with the summer outbreaks were isolated from two persons. It turns out these two cases occurred in siblings of an older child who had acquired the infection during the outbreak period after ingesting the beef.[74] Thus, salmonellosis in a community could be maintained by person-to-person transmission after introduction into that community by a contaminated product. The plasmid profile analysis, thus, helped not only to estimate an attributable fraction of a risk factor but also to describe the dynamics of community transmission of salmonellosis that would have been difficult, if not impossible, to do by conventional epidemiologic methods.

4.3. Stratifying Data to Refine Epidemiologic Study Designs

Stratification of data is one of the most common analytical procedures used in epidemiology to reduce bias after data had already been collected. It helps to determine more precisely an association of an attribute with disease or outcome, controlling for the effects of a larger stratum to which that attribute belongs.[1] Because molecular strain typing methods can group data related to infectious agents by subtypes, the strain subtype information itself can be used to develop a new case definition, which may comprise one stratum.

For example, in a chest clinic in a large city, patients from whom *M. tuberculosis* is isolated would all be grouped as culture-positive tuberculosis (TB) patients. If one were to do a study to identify risk factors for TB, the initial case definition of TB may be patients from whom *M. tuberculosis* is isolated. Here, an analytical study to identify risk factor for TB is likely to identify host-related factors, such as immunosuppression, old age, diabetes, and poverty. However, if the *M. tuberculosis* isolates from different patients can be tested for drug susceptibility, then these TB patients may be reclassified, according to the drug resistance of their isolates. Such TB patients may then be regrouped into two new strata—those with multidrug-resistant TB (MDRTB) and those with drug-susceptible TB. Each patient stratum (MDRTB versus drug-susceptible TB) can then be used to define cases or controls or comparison group in a case–control analysis to identify a new risk factor (e.g., risk for MDRTB). Such an analysis will find risk factors that are more specific than the

risk factors identified for TB above. They may include, for example, previous history of TB treatment.

Then, if the MDRTB *M. tuberculosis* isolates from above are genotyped and the collection of these isolates are found to be comprised of one clone responsible for 40% of that collection, one can then look for risk factors for infection with that clone. Persons infected with that clone may share a common exposure, such as being exposed to a co-worker with TB at an office. The fact that 40% of them belonged to a cluster also suggests that the people infected with this clone were recently exposed and that they did not result from an infection that occurred many years ago.

One reported study illustrates this idea.[75] In the 1970s and 1980s in São Paulo, Brazil, multidrug-resistant strains of *Salmonella* serotype Typhimurium were a common cause of childhood diarrhea as well as meningitis.[76] The sources of these *Salmonella* organisms were unknown. In a multiclinic study of acute diarrhea in children less than 6 years of age in the city in the early 1980s, *Salmonella* were isolated from 40 (11%) of 358 children with diarrhea, 22 of which were *S*. Typhimurium; all were multidrug resistant. A case–control study comparing children infected with *S*. Typhimurium versus other serotypes of *Salmonella* found recent hospitalization to be a risk factor for *S*. Typhimurium infection. In the study, children who came to the study clinics with diarrhea developed diarrhea at home. Children with history of recent hospitalization were previously hospitalized for illnesses such as pneumonia and acute respiratory infections that required antimicrobial drug treatment. Because it was known at that time that recent exposure to penicillins or antimicrobial agents was a risk factor for acquisition of multidrug-resistant salmonellosis,[77] the investigators were faced with the question of whether the children with *S*. Typhimurium salmonellosis got infected at home or during the previous hospitalization.

The investigators examined plasmid profile of the *S*. Typhimurium isolates. They identified four distinct plasmid profiles that accounted for 47% of the 22 isolates (Figure 1). Each profile was found to cluster in time and in a particular hospital in the city. The others spread over a 2-year period in a typical endemic pattern of occurrence. Thus, 47% of the *S*. Typhimurium infections could be traced to particular hospitals in the city.[77] Furthermore, the study showed that what appeared to be an endemic pattern of disease occurrence in a community was actually composed of at least four outbreaks that occurred in hospitals, unrecognized until the isolates were genotyped.

A similar observation was made by a more recent study using PFGE to subtype *S*. Typhimurium isolates from "sporadic" cases of salmonellosis in Minnesota between 1994 and 1998.[78] Of 174 distinct PFGE patterns found among 958 isolates, the investigators detected four large outbreaks in the state that had not been previously recognized.[78] They also identified many multidrug-resistant strains that had been previously described among *S*. Typhimurium isolates obtained by CDC in the 1970s–1980s.

Thus, in the above two examples of salmonellosis, strain typing analyses (plasmid profile or the PFGE strain typing) helped to "unmask" several discrete outbreaks from a background of so-called sporadic or "endemic" cases of salmonellosis in a city and state. Endemic salmonellosis appears to be comprised of many small outbreaks. This is one of the major discoveries about infectious disease epidemiology made in the last 25 years by the application of molecular biology tools. In fact, it challenges the traditional view about infectious disease occurrence and raises a rather provocative suggestion that there is no such thing as endemic salmonellosis; all cases of salmonellosis may actually be part of outbreaks that go undetected because of absence of strain type information.

Thus, as illustrated above, different laboratory tests identified distinct strata, each of which then could be used to define a new group (i.e., new case definition). Then each stratum could be compared against others in a case–control study design to identify a risk factor for one group. Genotyping data provide the most refined stratum, and if the sample size does not get too small, an analysis can be performed to identify a specific risk factor for that stratum. More refined the stratum, more specifically the risk factor for that stratum is identified. Furthermore, stratification by genotyping methods identifies risk factors related to the pathogen itself—specific site of transmission of a specific clone in the example of salmonellosis in São Paulo, Brazil. Thus, genotyping creates an opportunity to do an analytical study that generates a new hypothesis—refines a study design.

Most importantly, from a public health perspective, a study design that can identify risks for a refined stratum can also identify a more focused intervention option. Stratification of cases, for instance, by antibiograms of the case isolates usually identifies host-related factors as risk factors—e.g., previous exposure to antibiotics. This knowledge, while helpful, does not provide a focused intervention option, and often, as we all know, such knowledge does not contribute to long-lasting effective interventions. In the example of salmonellosis in Brazil, however, further stratification of the *Salmonella* isolates by plasmid profile analysis identified a more specific risk factor related to the isolates themselves. The knowledge that many cases of multidrug-resistant salmonellosis were occurring in specific hospitals in the city brought attention to the need to identify factors within the hospitals that contribute to *Salmonella* transmission. Today, nosocomial transmission of multidrug-resistant

salmonellosis in São Paulo is rare. Thus, this ability to create an opportunity to identify specific intervention options is another major contribution to infectious disease epidemiology made by molecular epidemiology. Many examples of such applications are described elsewhere.[1]

4.4. Distinguishing Pathovars from Nonpathovars

E. coli can cause diarrhea, urinary tract infection (UTI), meningitis, septicemia, septic shock, and wound infections. Yet there are *E. coli* strains that colonize our intestine without ever causing disease. Among *E. coli* strains that cause diarrhea, there are several distinct groups, and they differ from those that cause UTI, meningitis, septicemia, and other diseases. Thus, being able to distinguish *E. coli* strains associated with a particular type of disease, and separating them from non-pathogenic variants of *E. coli*, is an important activity in epidemiologic investigation. This way of classifying strains is called virulence or pathogen typing. *E. coli* strains that cause disease are the best examples of human *pathotype* or *pathovar*.

While traditional methods are also available to separate *E. coli* into distinct virulence types, molecular biology techniques have greatly simplified this task. In particular, *E. coli* strains associated with diarrhea have come to be classified into distinct groups (enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enterohemorrhagic *E. coli*, shigatoxin-producing *E. coli*, enteroaggregative *E. coli*, diffuse-adherent *E. coli*), based on a unique set of factors that define the group.[79] However, it should be noted that what made possible the attribution of these virulence factors to these groups of *E. coli* is that many of these groups have been recognized to cause outbreaks, either at the community level or in institutional settings.

In obvious outbreaks, it is not necessary to genotype the implicated pathogen to claim that there was an outbreak. In fact, it is the other way around. Outbreaks are used to validate a genotyping test itself. If a particular genotyping method shows that a strain maker is indistinguishable in all the isolates from an outbreak, but that it differs from comparison isolates obtained from sources unrelated to that outbreak, that method is said to be validated for epidemiologic investigation of that organism. That is, the method can be used to study apparent sporadic diseases caused by that organism.

However, there is another group of *E. coli* that causes extraintestinal infections (sometimes referred to as ExPEC, or extraintestinal pathogenic *E. coli*), which are not as obvious as to why they cause disease.[80] That is, it is not clear whether these *E. coli* strains are members of the normal intestinal flora that cause disease only when they happen to enter a sterile niche, such as the blood stream, bladder, CSF, or deep tissue. Unlike the groups of *E. coli* that cause diarrhea, ExPECs do not carry any virulence factors that clearly distinguish them from those strains isolated from the intestine. Unlike diarrheagenic *E. coli*, ExPECs are not usually recognized to be associated with obvious outbreaks. This makes attribution of a clone of ExPEC to disease more difficult.

Thus, with ExPECs, the application of genotyping methods to address their epidemiology uses an approach distinct from that used to study diarrheagenic *E. coli*. A recent set of studies of community-acquired urinary tract infections (UTI) suggest that outbreaks of UTI do occur in community settings.[81–84] Such observation was used by investigators to postulate that some of the multidrug-resistant uropathogenic *E. coli* strains causing UTI in women may have food animal as a reservoir.[81, 85–87]

In diseases such as UTI where an outbreak occurrence is not obvious, a genotyping method itself may provide a clue that an outbreak may have occurred in a community; this observation generates a new hypothesis about disease transmission. The hypothesis, then, can be tested by conducting a case–control or cross-sectional study. For example, with UTI, a study can be performed to see if there is any association between a predominant clonal group of uropathogenic *E. coli* identified by a strain typing method and a risk factor. If so, this would provide evidence that there was indeed an outbreak. Here, the ultimate confirmation of the hypothesis will be made by showing that the removal of that risk factor leads to amelioration of the recognized problem.

Same questions arise in distinguishing organisms that also happen to colonize non-sterile niches of the human host or occur as saprophytes in the environment. Examples of the latter include *S. pneumoniae*, *Helicobacter pylori*, *S. aureus*, and Gram-negative bacteria other than *E. coli*. Approaches similar to those used to study ExPECs can be applied for these organisms.

4.5. Addressing Hospital and Institutional Infectious Disease Problems

Hospital-associated or nosocomial infections are defined elsewhere in this book (See Chapter 26). In developed countries, organisms that are most frequently associated with nosocomial infections are usually members of the "normal" flora in the non-sterile niche of a human host, which include the skin, nasopharynx, and colon, or saprophytic organisms in the hospital environment. The most common such agents of hospital infections include coagulase-negative *Staphylococci, S. aureus, Enterococcus spp., Clostridium difficile, E. coli, Enterobacter spp*., *Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter spp., Proteus spp.,*

Stenotrophomonas maltophilia, Serratia spp., and *Candida spp*. Non-commensal or non-saprophytic pathogens that cause hospital infections include those transmitted by blood products (e.g., HIV, HBV, HCV, *Yersinia enterocolitica*) and respiratory pathogens (including severe acute respiratory syndrome [SARS]-associated coronavirus). In developing countries infections caused by *M. tuberculosis* and *Salmonella* introduced into a hospital by an index case, staff, visitors, or contaminated food are still common.

Genotyping bacterial organisms isolated in hospital or other institutional settings have become routine in many places, especially in developed countries. In fact, these techniques have become an indispensable part of hospital infection control activities.[88] While probe-based methods are most frequently used to detect drug-resistant organisms to augment clinical management of patients, many hospitals use genotyping tests to support nosocomial infection control practices. Teaching hospitals use genotyping methods to study nosocomial infection epidemiology.

Many of the infectious disease epidemiologic concerns in institutional settings are distinct from those related to field or community settings. While obvious outbreaks do occur in hospitals, very often, clusters of infectious diseases observed in hospitals are difficult to characterize as outbreaks. This is because patients in hospitals have host-related factors (immunosuppression, prolonged antimicrobial drug exposure, underlying illness) that predispose them to infection with "normal flora", saprophytes, or recognized pathogens. The distinction among colonization, contamination, and infection becomes blurred and is a frequent topic of discussion among hospital staff. One important question that arises in these settings, therefore, is, "do these organisms have biologic characteristics that enable them to cause disease or do they cause disease only because of host susceptibility factors?"

Another major area of concern in hospital infections, of course, is the drug-resistant infections. Very often, when risk factor studies for drug-resistant infections in hospitals are carried out, they find host-related factors—antimicrobial drug exposure, old age, underlying medical conditions, prolonged hospital stay, etc. Conventional epidemiologic studies rarely identify factors that can be attributed directly to the organisms themselves—sources or reservoir, transmissibility, and their biologic characteristics that determine disease production versus asymptomatic colonization. Thus, in hospital epidemiology, another important question is, "do antimicrobial drug-resistant microorganisms predominate in hospital settings because they are selected by the use of antimicrobial agents, or do they predominate because of distinct biologic characteristics possessed by a limited number of clonal groups that happen to be drug resistant?" Molecular epidemiologic methods are beginning to address these questions.

Given that normal flora and saprophytes found in hospital settings are comprised of a large number of strain types, one would expect drug selection pressure to generate a large number of drug-resistant strains to cause hospital infections. One of the most intriguing observations made recently about drug-resistant bacterial hospital infections, however, is that, despite strong antimicrobial drug selection pressures, most of these infections are actually caused by a limited set of bacterial strains. Most studies found that drug-resistant strains that are isolated from hospital invasive disease sources are comprised of a limited number of strains. In New York, a 2-year survey of vancomycin-resistant *Enterococcus faecium* (VRE) infections at a teaching hospital found 54 different PFGE subtypes among 182 isolates. One-third of these were accounted for by just two PFGE subtypes.[89] In Tennessee, a similar survey in different hospitals over a 3-year period showed that among 34 different PFGE types, one was responsible for 61% of 106 VRE isolates obtained from seven different hospitals.[90] In Tennessee, the investigators found that the introduction of the predominant PFGE types at different institutions was caused by new patients transferred to these institutions. Thus, these studies suggest that a clonal strain carried by a transfer patient, once introduced into a hospital setting, can rapidly become predominant in that hospital, and that such a mode of transmission is more important than the selective pressures of antimicrobial drugs in establishing a large number of drug-resistant nosocomial infections. Without the genotype data, such a conclusion would be difficult to make. This type of observation also helps to streamline nosocomial infection control practices. In this case, the recommendation based on the observation is to screen transfer patients for VRE before they are sent to a particular ward in the recipient hospital.

The observations described above with VRE apply to other hospital infections, including those caused by methicillin-sensitive (MSSA) or methicillin-resistant *S. aureus* (MRSA), *E. coli*, and other Gram-negative bacteria. It is beyond the scope of this chapter to discuss each of these agents of hospital infection, and the reader is encouraged to refer to reference (1) for further reading.

4.6. Identifying Genetic Determinants of Disease Transmission

The next stage in the evolution of laboratory-based epidemiology is the methodology to identify genetic basis for disease transmission. This, of course, applies to both the host and the pathogen. Host factors that enhance the

likelihood of development of a clinical manifestation after a bacterial infection could also render such hosts possible new sources of transmission to other hosts. Communities with a high prevalence of persons with HIV infection or AIDS facilitate greater likelihood of transmission of tuberculosis, just because of the greater prevalence of cases of tuberculosis. Thus, host genetic factors that have a similar effect on infection could contribute to enhanced transmission of a disease. Studies that examine host genetic factors that affect disease transmission are just beginning.[91] Of course, in addition to host factors, environment can greatly influence infectious disease distribution, including temperature, weather, season, urbanization, industrialization, and socioeconomic conditions. Such influences on disease transmission and epidemiology of infectious diseases require a separate chapter in itself. In this chapter, only pathogen genetic factors that facilitate transmission and therefore disease distribution are dealt with.

As mentioned earlier, one of the common molecular epidemiologic exercises involves attempts to explain over-representation of clones, clonal groups, or clonal complex strains in a community or institutional settings. Many of these can be explained on the basis of epidemiology, such as point-source contamination of a widely distributed food product (as in salmonellosis) or airborne transmission from a single index case (as in tuberculosis). However, we also know of many situations in which there is no obvious explanation for the predominance of one particular strain of a microorganism. Once epidemiologic explanation for the predominance is ruled out, possible biologic bases for this observation needs to be examined.

All pathogens have developed their own distinct strategies to transmit themselves to new hosts within a context of an environmental setting. Studies to date that have attempted to identify a pathogen's specific genetic determinants of transmission have used two different approaches: (1) those that attempt to identify a type of infection in a host that enhances opportunities for transmission to new hosts and (2) those that attempt to identify a unique niche or reservoir that becomes a major source of transmission to human or other animal hosts.

Tuberculosis can arise from an infection that occurred in the remote past (reactivation disease) or from a recent new infection (rapidly progressive disease). As mentioned earlier, over-representation of a particular strain of *M. tuberculosis* in a community could result from recent transmission from a highly infectious index case (e.g., someone with a cavitary lung lesion working in an enclosed space with an opportunity for contact with many others). In a study conducted in the early 1990s in New York City, one drug-susceptible strain of *M. tuberculosis* called "C strain" was found to be responsible for nearly 10% of all newly diagnosed cases of tuberculosis.[92] A series of case–control studies found C strain to be significantly associated with injection drug use. However, there was no obvious epidemiologic relationship among the injection drug users infected with this strain, nor was there any epidemiologic relationship among non-users infected with the same strain. This prompted investigators to look for a biologic basis for the predominance of C strain in New York City.

Ultimately, C strain but none of the other *M. tuberculosis* strains identified in the city was found to be resistant to reactive nitrogen intermediates (RNI) and hydrogen peroxide in vitro.[92] These are effector molecules that *M. tuberculosis* is likely to encounter inside macrophages in a host after infection. It was hypothesized that since injection drug users, who are normally a high-risk group for tuberculosis, regularly inject themselves with a variety of antigens that stimulate their macrophages to express RNI and hydrogen peroxide, such a practice will eventually select for RNI and hydrogen peroxide-resistant strain of *M. tuberculosis* to emerge in that risk group. If such a strain infects a non-user, it will cause a rapidly progressive disease because such a host does not carry macrophages making RNI and hydrogen peroxide, which would otherwise control the initial infection with *M. tuberculosis*. That is, infection/disease ratio increases when one is infected with such a strain. Thus, more people will develop rapidly progressive disease in a community where such a strain is circulating. This would explain the predominance of C strain in New York City. Hence, in this case, a human behavior appears to have selected for a new strain of *M. tuberculosis* that eventually affected the epidemiology of tuberculosis in New York City.

C strain was further studied in the laboratory and ultimately, a gene called *noxR1* was identified that when transferred into RNI-susceptible *E. coli* or *M. smegmatis* strains rendered these hosts resistant to RNI.[93] Subsequently, additional genes that exhibited RNI and hydrogen peroxide resistance were identified,[94, 95] which opened a new area of tuberculosis pathogenesis research—study of the mechanisms of resistance to natural antimicrobial effector molecules elaborated by the human host against *M. tuberculosis*. These genes may not completely explain the mode of transmission and reason for the predominance of *M. tuberculosis* strain in a community, but these are the earliest studies that applied molecular epidemiologic methods to identify a particular clone of a clinical isolate to study its biologic basis for its distribution and occurrence in a community. Here, the over-representation in New York City of an *M. tuberculosis* strain was attributed to this strain's enhanced ability to cause rapidly progressive disease after a new infection. A mechanism by which an *M. tuberculosis* strain can cause rapidly progressive disease was sought; the strain's resistance to macrophage effector molecules was found to be

possibly related to this mechanism. Genes that mediate this phenotype were identified.

The second type of approach used to identify a genetic basis for disease transmission focused on salmonellosis caused by *S. enterica* serotype Enteritidis. *S.* Enteritidis is the most common cause of egg-associated salmonellosis in developed countries. In a study of henhouses in the United States, 18–25% of captured mice were shown to be infected with *S.* Enteritidis, while *S*. Typhimurium was cultured from only 1.5% from the same henhouses.[96] In the same study, chicken eggs contaminated with *S.* Enteritidis were found in 9 (43%) of the 21 henhouses.[96] Unlike the other *Salmonella* serotypes that infect the surface of egg shells, *S*. Enteritidis infect eggs internally.[97] *S.* Enteritidis multiply inside the egg if left at room temperature. Thus, eggs serve as an "amplifier" of transmission to human hosts, which explain the unique epidemiology of salmonellosis caused by this pathogen. Thus, this second approach to identify a genetic basis for disease transmission attempted to look for a biologic mechanism for the establishment of a reservoir for a pathogen that then serves as a major source for human infection. The question, therefore, is, what enables *S*. Enteritidis and not the other *Salmonella* serotypes to survive in the egg?

This question was addressed by Lu et al., who examined multiple clinical isolates of *S*. Enteritidis for susceptibility to chicken egg albumen. They first found that most clinical isolates of *S*. Enteritidis were relatively more resistant to egg albumen than most clinical isolates of *S*. Typhimurium.[98] One *S*. Enteritidis strain that was highly resistant was analyzed further. Lu et al. eventually identified a gene called *yafD* that when transferred into an egg-susceptible strain of *S*. Typhimurium rendered it resistant.[98] Disruption of *yafD*in the original resistant *S*. Enteritidis strain made it more susceptible to egg albumen. The mechanism of resistance to albumen associated with *yafD* is not yet known.

Thus, once again in this example, a molecular epidemiologic approach first identified a clinical strain of *S*. Enteritidis, which when tested further for a distinct phenotype led to the identification of a gene associated with that phenotype. These types of studies should yield new understanding of how infectious disease transmission occurs and should provide novel approaches to disease control and prevention. These are just beginning to be applied in this new phase of molecular epidemiology research.

5. Summary

Molecular epidemiology should not be regarded as just another technique or a tool, but as an established discipline in epidemiology that can advance our knowledge about infectious diseases in ways that were not previously possible. Molecular epidemiology has begun to challenge old notions and reveal new paradigms concerning the epidemiology of infectious diseases. This chapter introduced new concepts that emerged within this discipline and a sample of its applications. The literature is vast and molecular epidemiologic methods are now a major component of most laboratory-based infectious disease epidemiologic studies. However, it is a constantly evolving discipline and should continue to provide new insights into elucidating epidemiology of infectious diseases in the future.

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CHAPTER 4

The Epidemiology of Bacterial Resistance to Antimicrobial Agents

Fred C. Tenover and John E. McGowan, Jr.

1. Introduction

Infectious diseases have continually played a role in shaping human history. The plague pandemic of the 13th century claimed approximately 25% of the world's population. Dysentery decimated the forces of Napoleon, and tuberculosis, also known as "the white plague," condemned many of history's most influential leaders, writers, artists, and scientists to untimely deaths, often in their most productive years. Thus, it was assumed that with the introduction of antimicrobial agents into clinical medicine during the 20th century, the conquest of infectious diseases was likely to be close at hand. Yet, a closer examination of the medical literature beginning in the 1940s reveals an ominous trend that would be repeated over and over again throughout the next several decades: The discovery of virtually every new antimicrobial agent effective against bacteria was followed sooner or later by the recognition of bacterial resistance to the agent. How bacteria develop resistance to antimicrobial agents and how resistant microorganisms spread in hospitals, other healthcare settings, and communities will be the focus of this chapter.

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2. Historical Background

Even while the wonder drug penicillin was making headlines by providing dramatic cures for patients with puerperal sepsis, gonorrhea, and other illnesses, strains showing resistance to the drug were already becoming apparent. Both gram-negative and gram-positive bacteria contained enzymes capable of hydrolyzing penicillin and destroying its antibacterial action.[1,2] Similarly, although the mechanisms of resistance were not understood in the 1940s and early 1950s, physicians treating patients with either of the new antituberculosis drugs, para-aminosalicylic acid or streptomycin, noted that after initial signs of improvement, some of their patients began to deteriorate clinically. The *Mycobacterium tuberculosis* strains recovered from the patients who failed therapy were now resistant in vitro to the antimicrobial agents they were given; this problem persists today.[3] Today, there are no antimicrobial agents used in human medicine for which resistance in at least one target organism has not been recognized.

The paradigm for antibacterial agents is that resistance follows use, and widespread use or misuse of a drug will eventually lead to its loss of effectiveness for treating human illness.[4,5] In some cases, resistance appears quickly. For example, resistance to the semisynthetic drug methicillin developed in *Staphylococcus aureus* just a few years after its introduction.[6] However, for other drug–organism combinations, such as vancomycin and *Enterococcus faecium*, almost 30 years passed between the introduction of the drug into human use and the emergence of resistance.[7] The reasons for the differences in time frames are unclear and probably multifactorial. Yet, the ability of bacteria to circumvent the

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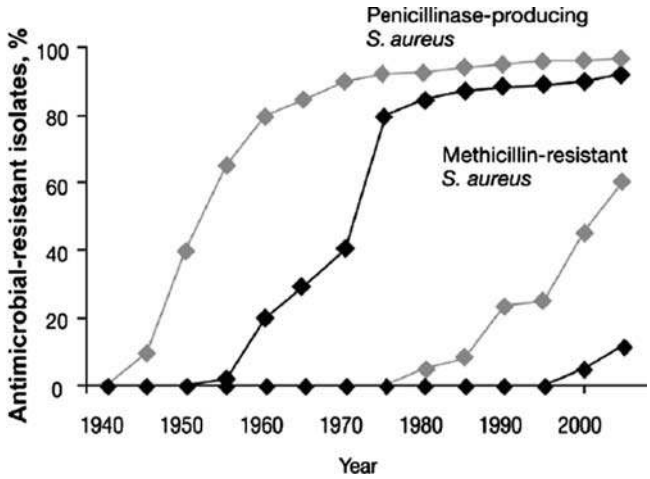


Figure 1. Evolution of antimicrobial-resistant *Staphylococcus aureus* as a cause of nosocomial and then community-acquired infections [5–7]. *Black squares,* nosocomial infection; *gray squares,* community-acquired infection. [From reference[9]]

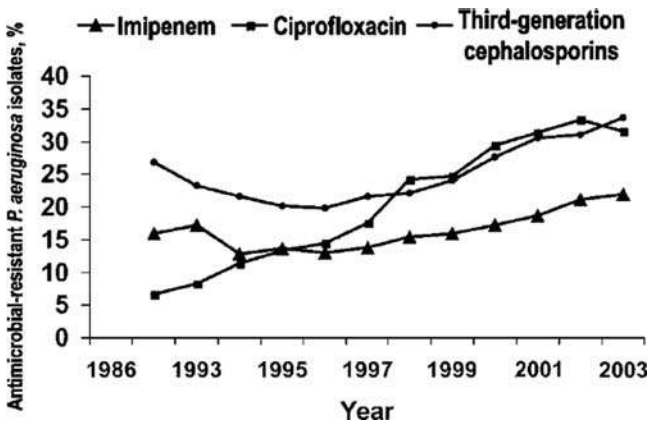


Figure 2. Resistance to antimicrobials (imipenem, ciprofloxacin, and third-generation cephalosporins) in *Pseudomonas aeruginosa* isolates recovered from intensive care unit patients (National Nosocomial Infections Surveillance System, unpublished data). [From reference[9]]

killing action of antimicrobial agents has clearly impeded our ability to treat individual patients and to control large outbreaks of infectious diseases.[8] Examples of emerging antimicrobial-resistance problems that have developed during the last two decades are given in Figures 1 and 2.[9]

2.1. Importance of Changes in Resistance Epidemiology

The continual spread and changing patterns of antimicrobial resistance in bacteria are of concern to clinicians and epidemiologists alike.[10,11] The increase in bacteria that are resistant to frequently used anti-infective agents is well documented.[9,12] Some health-care-associated organisms are resistant to all commonly used antimicrobial agents.[13,14] Whereas resistant bacteria were previously common only in intensive care units of hospitals, these health-care-related organisms more recently have been recovered from non-intensive care hospital inpatient areas and are now isolated with increasing frequency in extended care facilities, ambulatory surgical units, home health-care sites and other health-care settings.[15,16] Resistance also remains important in the "community" setting, that is, in situations where the victim has had no contact with health-care institutions.[17] To complicate matters even further, resistant organisms that arise in the community are now also spreading into health-care settings.[18,19]

3. Mechanisms of Resistance

3.1. Intrinsic Resistance

Some bacteria are intrinsically resistant to antimicrobial agents because (1) they lack the target site for that drug, (2) the drug is unable to reach the site of action, or (3) the organism contains a chromosomally encoded resistance mechanism (Table 1). For example, most gram-negative organisms are resistant to vancomycin because this large, hydrophobic drug cannot penetrate the organism's cell membrane.[20] Thus, these organisms are intrinsically resistant due to a permeability factor. By contrast, *Klebsiella pneumoniae* strains are resistant to ampicillin and other penicillins because these organisms contain a chromosomal β-lactamase that inactivates these and similar drugs.[21] Thus, intrinsic resistance may be due to a number of different mechanisms.

3.2. Acquired Resistance

Bacteria that are by nature susceptible to an antimicrobial agent may become resistant by chromosomal mutation or by the acquisition of new genetic material that encodes the proteins responsible for the resistance phenotype. Chromosomal mutations that occur within the genes that encode the target sites for antimicrobial agents may change the structure of the proteins enough to prevent binding of the drug. Streptomycin resistance in *M. tuberculosis* is a good example of this.[22] Furthermore, the accumulation of point mutations in several different key genes in *M. tuberculosis* under the selective pressure of inadequate antimicrobial therapy (often a result of patients not consistently taking their medications) probably resulted in the multidrug-resistant strains of tuberculosis that caused outbreaks in several large cities in the United States in the early 1990s.[23] Similar reasons

Table 1. Examples of Bacterial Mechanisms of Antimicrobial Resistance

Antimicrobial class	Example	Mechanism	Organisms
Aminoglycosides	Gentamicin	Drug modification	Enterobacteriaceae *Pseudomonas* Enterococci Staphylococci
	Amikacin	Reduced permeability	*Pseudomonas*
β-Lactam drugs	Penicillin	β-Lactamase	Staphylococci Enterococci Enterobacteriaceae *Pseudomonas* Gonococci
β-Lactam drugs	Penicillin	Altered cell wall	Pneumococci Meningococci
Fluoroquinolones	Ciprofloxacin	Altered DNA gyrase	Staphylococci *Pseudomonas* Enterobacteriaceae
Glycopeptides	Vancomycin	Altered DNA ligase	Enterococci
Macrolides	Erythromycin	RNA methylase	Staphylococci Pneumococci Streptococci
Tetracyclines	Doxycycline	Drug efflux	Streptococci Enterobacteriaceae Staphylococci *Pseudomonas*
Trimethoprim	Trimethoprim	Altered enzymes	Enterobacteriaceae *Pseudomonas*

Adapted from Neu.[20]

probably underlie the current prevalence of *M. tuberculosis* strains labeled XDR (extremely drug resistant).[3]

3.3. Molecular Mechanisms

Understanding the acquisition of resistance by bacteria requires consideration of the general pathways for appearance or spread of resistant bacteria in health-care and community settings, and an examination of the molecular mechanisms at work. Several resistance mechanisms have had a major impact on the resistance of gram-positive hospital organisms including new dihydrofolate reductases, novel changes in penicillin-binding proteins, mutations in topoisomerase genes, variants of aminoglycoside-modifying enzymes (which have become widely prevalent in enterococci), and new enzymes that alter cell wall composition.[24,25] These arise from duplication and diversification of existing genes, acquisition of new genes from other organisms, and mutation of acquired genes. The ability of pathogenic bacteria to acquire and disseminate these resistance determinants is orchestrated by a variety of plasmids, bacteriophages, transposons, and integrons.[24]

3.4. Epidemiologic Pathways

On a larger scale, several pathways have been described for appearance or spread of resistance in bacteria.[26] Each of these pathways can be associated with factors increasing risk of developing resistance[27] (Table 2). Recent investigations have highlighted the role of these pathways in clinical practice.

3.4.1. Introduction from Outside. New strains can be brought into a new setting from an outside source by way of a patient, a carrier, or a contaminated commercial product. For example, introduction of resistant strains into hospitals from nursing homes and extended care facilities, as well as transfer in the opposite direction from hospital to nursing home, has become common for certain pathogens.[28] Likewise, methicillin-resistant strains of *S. aureus* (MRSA) that have arisen in the community now have become prevalent in the hospital setting as well.[19]

3.4.2. Exchange of Resistance Determinants. Resistance can be acquired by a previously susceptible strain from another species or genus.[27] Genetic mutation and transfer of genetic material can produce resistance. Changes in only a few base pairs in chromosomal structural or

Table 2. Introduction and Spread of Antimicrobial Resistance into a New Setting

Problem	Example
Introduction of new resistant organisms from outside sources	MRSA from nursing home patient
Mutation of susceptible endemic organisms that leads to a resistant phenotype	Ciprofloxacin-resistant *P. aeruginosa*
Acquisition of new DNA by susceptible endemic organisms that results in resistance	Vancomycin-resistant enterococci
Emergence of inducible resistance in organism that carry previously silent resistance genes	Ceftazidime-resistant *Enterobacter*
Selection of a resistant population of organisms from a heteroresistant population	Aminoglycoside-resistant *Acinetobacter*
Spread of endemic resistant organism	*Serratia marcescens* in ICUs

Adapted from McGowan and Tenover.[27]

control genes may result in one or more amino acid substitutions in a crucial target (enzyme, cell structure, or cell wall), leading to new resistant strains. The change, although subtle, may inactivate whole groups of antimicrobial agents.[29]

3.4.3. Emergence or Selection. Chromosomal determinants for resistance to a given drug may not be expressed until the organism comes into contact with that antimicrobial agent or similar compounds.[27] When permissive conditions appear (e.g., new antimicrobial agents in use in a hospital or the introduction of new conjugative plasmids into a bacterial species), resistance can be manifested rapidly. For example, multiresistant strains of *Pseudomonas aeruginosa* rapidly emerged in patients in a stepwise fashion after exposure to antipseudomonal antimicrobials, and these strains were associated with adverse outcomes.[30] The trigger for emergence may be the antimicrobial agent to which resistance is directed or to another antimicrobial agent that results in induction or derepression of a determinant (enzyme, etc.) and stimulates resistance to the studied drug as well.[24]

Exposure to a stimulus that inhibits or kills the susceptible majority of a population allows a resistant subset of strains to grow, outcompeting the susceptible organisms. The factor in this selection process usually is the antimicrobial agent to which the subpopulation is resistant, but on occasion nonantimicrobial factors, such as those stimulating activity of reactions like acetylation or glucuronylation, also can provide a selective advantage to organisms. These mechanisms necessitate emphasis on avoiding careless use of antimicrobial agents, which can provide a trigger or stimulus to the development of these types of resistance.[31]

3.4.4. Clonal Dissemination. Organisms can spread in clonal fashion from patient to patient, from one patient to another via a health-care worker (e.g., on the hands of ward personnel), in contaminated commercial products (e.g., antiseptics), on other inanimate objects, or by widespread transfer of genetic material from the initial organism to others.[12,32]

3.4.5. Combinations of Pathways. Any or all of these transmission pathways conceivably may have a role in the appearance or increase of resistant organisms in the acute care setting and other health-care settings, such as the nursing home and ambulatory care unit. Sorting out these multiple and concurrent elements, which leads to the appearance and spread of resistance, is an important but difficult problem.[27]

3.5. Examples of Resistance Mechanisms for Various Classes of Antimicrobial Agents

3.5.1. β-Lactam Agents. Resistance to penicillins, cephalosporins, and carbapenems agents most often results from the presence of enzymes in the bacterial cell (β-lactamases) that hydrolyze the β-lactam ring of the antimicrobial agent, an action that detoxifies the drug.[26] However, β-lactam resistance can also be mediated by changes in the site of action of the drugs, the penicillin-binding proteins (PBPs).[33] This occurs by remodeling of the genes that encode the PBPs through incorporation of foreign DNA acquired by the bacteria most often through transformation. These novel mosaic genes containing DNA from the host organism and DNA acquired from related organisms produce altered, albeit functional, PBPs. The latter mechanism is particularly important in the development of penicillin resistance in *Streptococcus pneumoniae*.[34] Newer beta-lactamases have molecular changes that extend their activity to produce resistance to broad-spectrum beta-lactams like extended-spectrum cephalosporins[35] and carbapenems.[36,37]

3.5.2. Macrolides, Azalides, Lincosamides, and Streptogramins. The macrolides, which include agents such as erythromycin and clarithromycin, and the azalides, such as azithromycin, are commonly administered oral drugs used for the treatment of many bacterial respiratory infections and superficial skin infections. Resistance is due either to inactivation of drug (mediated by erythromycin esterases or phosphorylases), efflux of the drug out of the cell, or by modification of the site of action.[38] The latter mechanism, in which the 23S RNA of the 50S ribosome unit is methylated at a specific adenine residue, which prevents binding of the antimicrobial agent to the ribosome, not only leads to high-level resistance to macrolides but

also affects lincosamides (such as clindamycin) and streptogramins (such as pristinamycin), since all three classes of drugs act by binding to the same site on the bacterial ribosome. The so-called MLS-resistance phenotype (for macrolide–lincosamide–streptogramin) is typically observed in staphylococci and streptococci and is often plasmid mediated, but the genes encoding these enzymes also may be found on transposable elements.

3.5.3. Aminoglycosides. Aminoglycosides are commonly used in conjunction with β-lactam agents to treat serious bacterial infections, such as sepsis and endocarditis, because the two groups of drugs frequently act synergistically. Resistance to aminoglycosides is typically mediated by enzymes that modify the drug, so that uptake into the bacterial cell is impeded. However, efflux of drug out of the bacterial cell and permeability barriers to aminoglycosides are also recognized.[39] There are three types of aminoglycoside-modifying enzymes: those that acetylate, adenylate, or phosphorylate the drug. The number of genes encoding variants of these enzymes within each group is remarkably large and diverse. There is indirect evidence for two unique hypotheses on the origin of these resistance genes.[40] On the one hand, several of the genes are similar to those found in soil bacteria that produce antibiotics that protect the host organism from self-inhibition. The DNA encoding these genes may have been released into the soil through cell lysis and picked up by other bacteria, which in turn may have incorporated the DNA into a resident plasmid, allowing it to be mobilized to other bacterial species. An alternate hypothesis suggests that the proteins that modify the aminoglycosides have evolved from similar types of enzymes that have cellular "housekeeping" functions, such as phosphorylation of proteins to activate or inactivate enzymatic pathways.[40] These housekeeping enzymes became adapted over time to inactivation of aminoglycosides under the selective pressure of the presence of the antibiotics produced by other organisms in soil or in the hospital environment. Whether recruited from antibiotic-producing organisms or from other bacterial pathways, the array of aminoglycoside-resistance genes discovered to date is very diverse. The most recent example of this is the AAC(6')-Ib-cr acetylating enzyme that also inactivated ciprofloxacin and other fluoroquinolones that have a piperazine ring.[29]

3.5.4. Tetracycline. Tetracyclines are oral drugs that are frequently used for treating patients with a wide variety of community-acquired infections, including skin infections, Rocky Mountain spotted fever, and chlamydia. Resistance is mediated both by actively pumping of the drug out of the bacterial cell before it can reach its site of action and by target site alteration.[41] Tetracycline-resistance genes are frequently located on plasmids and transposable elements, which enhances their ability to disseminate throughout diverse species of bacteria. There are over a dozen such resistance genes that are divided into several subgroups based on DNA sequence and mechanism. Tetracycline-resistance genes are frequently found on genetic elements with other resistance genes, such as those mediating resistance to chloramphenicol. Many tetracycline-resistance mechanisms are not active against tigecycline, a new minocycline derivative.[42]

3.5.5. Trimethoprim and Sulfonamides. Trimethoprim and the sulfa drugs both inhibit the enzymatic pathway that synthesizes dihydrofolate.[43] Resistance is frequently the result of a plasmid-encoded enzyme that substitutes for a chromosomal enzyme normally inhibited by these drugs. Sulfonamides work through competitive inhibition of the enzyme dihydropteroate synthetase. The plasmid-encoded enzyme is smaller and more heat sensitive and requires a thousand times as much sulfonamide to inhibit it as does the chromosomal enzyme. Similarly, some strains resistant to high levels of trimethoprim, an agent that inhibits dihydrofolate reductase, contain a plasmid-mediated gene coding for a new trimethoprim-resistant dihydrofolate reductase. In each instance, the plasmids provide a mechanism whereby products vital to the bacterial cell can be synthesized and the inhibiting effect of the drug is bypassed.

3.5.6. Glycopeptides. Glycopeptide resistance can be mediated by a series of genes that effectively remodel the cell wall of an organism by altering the normal D-ala–D-ala binding site of vancomycin to D-ala–D-lac or D-ala–D-ser through introduction of an altered ligase enzyme (e.g., *vanA*).[44] The family of acquired vancomycin-resistance genes now includes *vanA*, *vanB*, *vanD*, *vanE,* and *vanG*.[45] The *vanA*-resistance determinant has been recognized among enterococci and *S. aureus* isolates, the latter due to acquisition of a transposon, Tn*1545* and its variants. A second mechanism of resistance noted among glycopeptide-intermediate *S. aureus* (GISA) strains is the thickening of the cell wall in conjunction with metabolic changes that make *S. aureus* isolates no longer susceptible to glycopeptides.[46,47] Such strains with reduced susceptibility to glycopeptides cannot be detected in the clinical laboratory using disk diffusion.[48]

3.5.7. Fluoroquinolones. Fluoroquinolone antimicrobials have been available worldwide for more than a decade.[49] Their widespread use has led to increasing prevalence of resistance to these drugs in most parts of the world. Resistance to fluoroquinolones typically arises by alterations in the target enzymes (DNA gyrase and topoisomerase IV) and through changes in drug entry and efflux.[50] Recent discovery of plasmid-mediated horizontally transferable genes encoding quinolone resistance (e.g., *qnrA*, *qnrB*,

and *qnrS*) perhaps explains some of the rapid emergence of resistance to these drugs.[51] Likewise, AAC(6')-Ib-cr, a variant aminoglycoside acetyltransferase capable of modifying ciprofloxacin and reducing its activity that was mentioned above, also seems to provide low-level quinolone resistance.[29] Each of these mechanisms may facilitate the emergence of higher level quinolones resistance.[51]

3.5.8. Oxalidinones. Resistance to the oxazolidinones, such as linezolid, among staphylococci and enterococci is due to modification of ribosomal RNA often through a G–T substitution at position 2,765.[52,53] Such strains usually have reduced fitness. Fortunately, this genotype is not transmissible to other strains.

3.6. Key Factors in the Development of Resistant Microorganisms

Three factors appear to be critical in the continuing evolution of resistant microorganisms: selection of mutations in common genes that extend their spectrum of resistance, transfer of resistance genes among bacteria, and selective pressures, primarily by antimicrobial use that enhance the development of spread of resistant organisms.[32] Programs for control and prevention of resistance must take heed of these factors.[54]

3.6.1. Selection of Resistant Mutations. The progression of simple β-lactam hydrolyzing enzymes, i.e., penicillinases and first-generation cephalosporinases, to the extended-spectrum β-lactamases (ESBLs), which hydrolyze not only penicillin-type drugs and first-generation cephalosporins, but extended-spectrum cephalosporins (such as cefotaxime, ceftriaxone, and ceftazidime) and monobactams (such as aztreonam), is an example of extending the phenotype of already existing resistance genes.[35] Such ESBL enzymes were first recognized among isolates of *K. pneumoniae* in Germany in 1982.[55] Mutations in the gene encoding the TEM-1 β-lactamase (named after the initials of the patient from whom the first ampicillin-resistant *Escherichia coli* was isolated) led to changes in three amino acids at key points in the enzyme. These changes enabled the active site of the enzyme to accommodate the much larger expanded-spectrum cephalosporins, which resulted in their inactivation. Further investigations of TEM β-lactamase mutants and of the mutants of a related β-lactamase, the SHV family of enzymes, have shown that even a single amino acid change can result in some ESBL activity.[26] The ESBLs now include variations of CTX-M enzymes (a group of cephalosporinase enzymes that hydrolyze the drug cefotaxime more rapidly than they do the drug ceftazidime). The presence of such enzymes in the United States and Europe appears to be increasing.[26]

3.6.2. Genetic Exchange Among Bacteria. Genetic exchange may occur by transformation, conjugation, or bacteriophage-mediated transduction.[40,56] For example, conjugal transfer of the staphylococcal β-lactamase[57] and aminoglycoside-resistance genes[58] to enterococci led to development of multiply-resistant strains that have proven very difficult to treat, particularly when coupled with vancomycin resistance.[16] Vancomycin-resistant enterococci, in turn, have become a significant problem in the United States, particularly in hospital intensive care units.[9] Six vancomycin-resistant *S. aureus* isolates have been recognized in the United States (CDC unpublished observations). The breadth of the genetic exchange network among microorganisms is only beginning to be understood.

3.6.3. Selective Pressure Arising from Antimicrobial Use. The final factor in the evolution of resistance is the broadening of the selective pressure in health-care and community settings that facilitates the emergence of resistance. The term *selective pressure* refers to the environment created through the use of antimicrobial agents that enhances the ability of an organism that becomes resistant by mutation or through acquisition of new DNA to survive and proliferate. Although resistance is not confined to hospital strains, the hospital has often been viewed as an important developing ground of resistant bacteria due to the extensive use of antimicrobial agents in this setting.[27] In addition to extensive use of antimicrobials in humans, the extensive use of antimicrobial agents among children for ear and respiratory infections,[59] and the widespread use of antimicrobial agents in agriculture and animal husbandry,[60] have enhanced development of novel resistance patterns in a variety of pathogenic bacteria in the community setting as well as in the hospital. Although the use of antimicrobial agents as feed additives have been banned in Europe,[61] the practice continues in the United States, South America, and other parts of the world.[62]

3.7. Mechanisms of Gene Dissemination

A key factor in the spread of resistance is the movement of genetic elements independent of the spread of bacterial strains. Figure 3 shows the ways in which an organism may become resistant to antimicrobial agents.[27]

Plasmids are extrachromosomal pieces of DNA that are not necessary for cell survival, but often give organisms a selective advantage to enhance survival. Plasmids may carry antimicrobial-resistance genes, and those plasmids may or may not be self-transmissible to other organisms via conjugation. Small, nontransmissible plasmids may still be mobilized to a bacterial recipient by larger conjugal plasmids present in the same cell. The transfer of

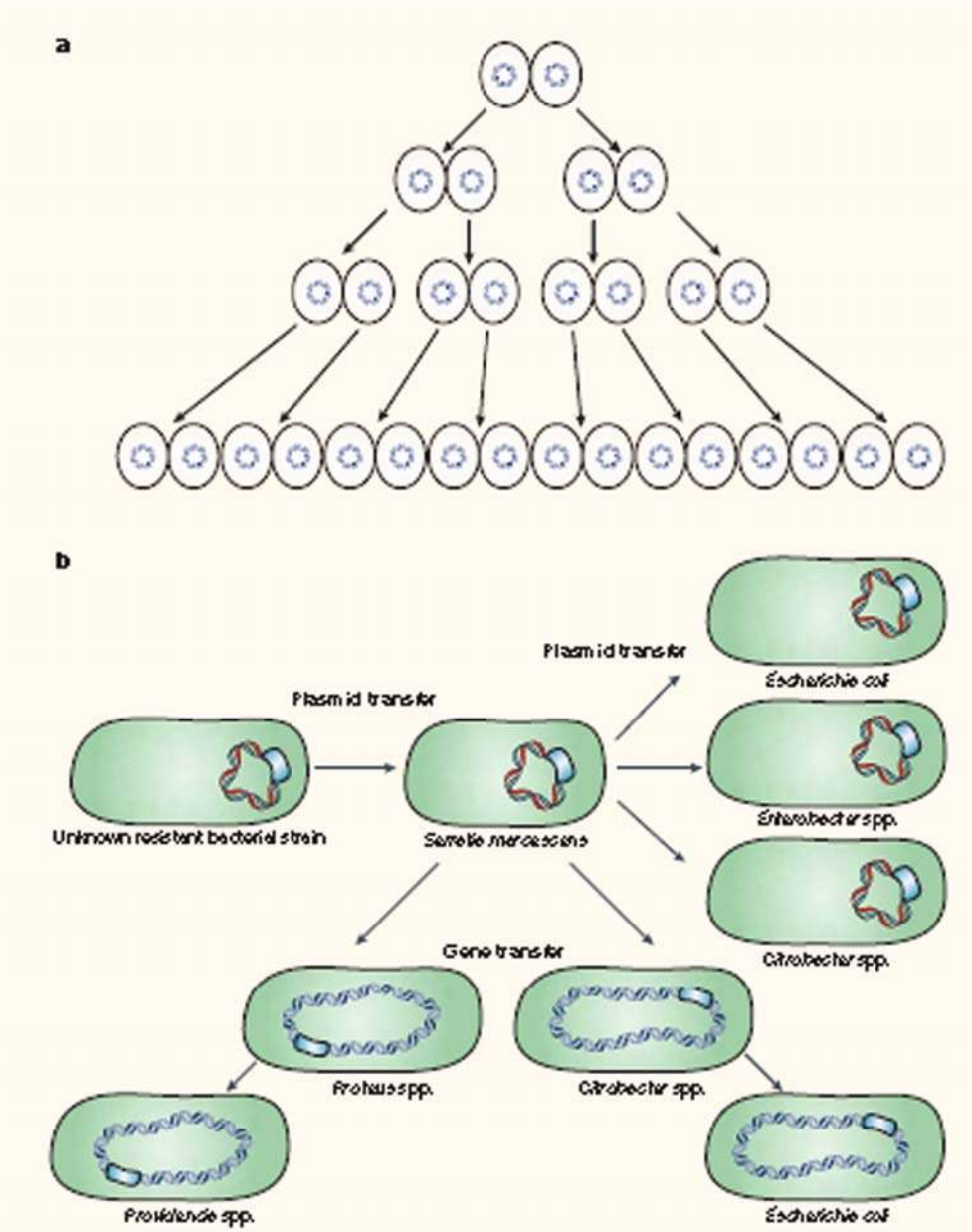


Figure 3. Mechanisms of spread of bacterial resistance. **A** Dissemination of vancomycin-resistant enterococcus carrying the *vanA* resistance gene on a plasmid (*inside circle*). This represents clonal spread of an organism in a hospital. **B** Dissemination of an aminoglycoside-resistance gene (indicated as *bar* on circular plasmid) by spread of the resistant strain, spread of the plasmid containing the gene, and spread of the gene on a transposable element. The plasmid containing the resistance gene was introduced from an unknown strain into an isolate of *Serratia marcescens,* which disseminated and spread among patients in the hospital. The plasmid then transferred to other members of the Enterobacteriaceae (*E. coli*, *Enterobacter,* and *Citrobacter*) via conjugation, followed by transposition of the resistance gene to other plasmids in *Proteus* and *Citrobacter*. The new plasmids containing the resistance gene were then transferred via conjugation to *Providencia* and other strains of *E. coli.* [From reference[27]]

resistance genes via plasmids was first noted in the 1950s by Japanese researchers who reported transfer of a multidrug-resistance phenotype from *Shigella* species to *E. coli*.[63] Plasmids have been reported to mediate resistance to as many as 12 antimicrobial agents,[64] and many plasmids are capable of being maintained by enteric bacilli, pseudomonads, vibrios, and other gram-negative organisms. One particular multiresistance plasmid, pLST1000, was isolated from

gram-negative pathogens in several US cities and from isolates from Venezuela. The mode of intercontinental transmission was not apparent, but the stability of the plasmid over time was remarkable.[65]

As the phenomenon of plasmid transfer among microorganisms was emerging, another genetic element was recognized in gram-negative organisms—the transposon. This genetic element, which was capable of moving from replicon to replicon, such as plasmid to chromosome or vice versa, moved independently of the typical functions of generalized recombination. One family of transposons, those similar to Tn*21*, were found in a variety of multiply-resistant enteric organisms in the late 1970s and 1980s.[66] Later, a set of genetic elements with a very similar structure was discovered in gram-positive organisms, but these elements were conjugal, i.e., the elements were able to move from organism to organism via cell-to-cell contact.[67] These conjugal transposons were observed first in pneumococci[68] and later in *Bacteroides fragilis*.[69] Since pneumococci, in particular, do not maintain plasmids, conjugal transposons probably played a major role in the development of multiresistance in this species. Conjugal transposons apparently require cell-to-cell contact and probably transfer via formation of a circular intermediate form.[67] Together with integrons, the elements into which resistance gene cassettes can enter with relative ease,[70] these elements serve to enhance the ability of resistance determinants to disseminate throughout bacterial populations at a rate much faster and more efficient than could dissemination via plasmids alone.

4. Epidemiology of Antimicrobial Resistance

Studying the epidemiology of bacterial drug resistance has become more of a challenge over the past 20 years as organisms have developed multidrug resistance and the genes encoding resistance have apparently become more mobile. However, newer molecular strain-typing techniques have enabled us to assemble a clearer picture of this process. [27]

4.1. Resistance in Health Care Contrasted with Community Settings

The acquisition of a bacterial pathogen can occur in the health-care setting or in the community, although the two ecosystems are closely linked.[19,27] Those infections that first occur in patients who have been a resident inside a hospital for more than 48 h are called health care associated, while other infections are deemed to be community associated.

For many bacteria that cause health-care-associated infections, the prevalence of resistant isolates varies by location in the institution (for example, between intensive care units (ICU) and non-ICU areas in hospitals)[71,72] (Figure 1). Patterns of resistance in *S. aureus* illustrate this point—data from Project ICARE, a cooperative surveillance effort in about 40 hospitals around the United States, show differences in the prevalence of MRSA isolates from ICU and non-ICU settings. It also has shown an increasing prevalence of MRSA in both ICU and non-ICU hospital areas during three time periods surveyed between 1996 and 2002[73,74] (Figure 2). The prevalence of resistance to selected antimicrobial agents in gram-negative aerobic bacilli also varies by health-care unit.[75] For example, the prevalence of resistance to third-generation cephalosporins in several gram-negative bacilli has increased dramatically throughout the United States in recent years[9] (Figure 3). Similar increases in resistance have also been documented among patients outside the hospital setting.[17] However, among community-associated infections, some still have links back to health-care settings such as with patients who have indwelling catheters or receive dialysis, or those who have been in a health-care facility in the last year.[76]

4.2. New Challenges Related to Resistance Epidemiology

The emergence of resistant bacteria has taken place over several decades. However, in the past 10 years, new facets of clinical resistance have emerged which have led to new challenges for microbiology service providers.

4.2.1. Alteration of Antimicrobial Use Patterns. The emergence of new patterns of antimicrobial resistance can alter the use of antimicrobial agents. For example, the emergence of vancomycin-intermediate *S. aureus* (VISA) strains (originally defined as strains with vancomycin minimal inhibitory concentration (MIC) of 8–16 μg/ml)—which were first detected in patients in Japan and then in the United States—was followed by reports of the isolation of two strains of vancomycin-resistant *S. aureus* (VRSA; MIC $\geq$32 μg/ml) from two different areas of the United States.[77–79] Detection of both VISA and VRSA strains has proved difficult in clinical laboratories using automated methods[80,81] because changes that lead to increased MICs are not readily detected by the analyses and interpretation algorithms that are used in many automated systems. If resistance to vancomycin among staphylococci continues to develop and spread, the use of vancomycin, which has long been considered the mainstay for the treatment of MRSA, could be severely compromised. Fortunately, other chemical

compounds now have been approved for use against MRSA, such as linezolid and daptomycin, and others are in later stages of clinical trials, or earlier stages of clinical development.[82]

4.2.2. Interference with Effective Treatment. During the past decade, strains of *Acinetobacter baumanii* and *P. aeruginosa* have arisen that are resistant to all the antimicrobial agents that are usually tested.[13] These organisms have become important hospital pathogens, especially in patients with hospital-onset pneumonia, so loss of the usual antimicrobial agents for treatment can dramatically interfere with patient care.[83,84] Increasing resistance in these organisms has resulted in treatment of patients with drugs such as colistin and polymyxin B, which were essentially abandoned in the 1970s because of their unacceptable levels of toxicity compared with alternative antimicrobial agents.[85,86] Unfortunately, few laboratories now have the capability to test whether organisms are susceptible to both these older antimicrobials, because appropriate testing materials are not widely available and the interpretation of test results is not straightforward.

Resistance to several different classes of antimicrobial agents continues to develop in organisms, such as *Acinetobacter*, with clinically significant, but difficult to detect, resistance phenotypes.[87] This trend fuels the demand for reliable, practical, and cost-effective detection methods for identifying strains that show new resistance patterns.

4.2.3. Complexity of Diagnosis. New patterns of antibacterial resistance can be quite difficult to detect.[88] This makes it necessary to develop new screening methods for detection of organisms like VISA, VRSA, and those containing ESBLs that are both practical and affordable for clinical and public-health laboratories.

The emergence of bacterial strains that are resistant to routine therapies increases the need for susceptibility testing of alternative therapeutic agents.[8] For example, routine testing of linezolid, quinupristin/dalfopristin, and daptomycin now is needed in areas where prevalence of VISA or VRSA strains is high, routine testing of colistin or polymyxin B might be added when *A. baumanii* strains are resistant to aminoglycosides, beta-lactam inhibitor combinations, and carbapenems.[87] When resistance precludes the use of standard drugs it can also increase the need for testing susceptibility to investigational drugs (that is, those that are not yet licensed in a given country or community), such as testing of MRSA or vancomycin-resistant enterococci (VRE) against dalbavancin.[89]

4.2.4. Altering Requirements for Surveillance. In addition to these local variations, changes in resistance patterns can affect whole cities, regions, or countries, and some changes are evident across national boundaries.[12,90] The appearance of VRE infections has been reported from such diverse parts of the world as North America and Western Australia.[91] This emphasizes the need for reliable surveillance systems that track both health-care-associated and community-based resistance, with local, regional, national, and international scope.[92] However, such systems would need to be standardized and validated to make results comparable.[93]

5. Organisms of Special Importance

5.1. Health-Care-Associated Pathogens

Two health-care-related pathogens continue to be of particular concern: MRSA and vancomycin-resistant enterococci (VRE). In some hospitals, over 70% of the *S. aureus* isolated from inpatients are MRSA,[9] and these strains often are resistant to all licensed drugs except vancomycin, linezolid, daptomycin, and tigecycline.[94] Recently, strains of *S. aureus* completely resistant to vancomycin were isolated from patients in the United States[95] further complicating therapy.[96] MRSA have become highly endemic in many hospitals, and in the community as well, which makes tracking of outbreaks more difficult. Once introduced into a hospital, this organism is very difficult to eradicate.[97,98] Problems with eradication are also true for vancomycin-resistant strains of *E. faecium*, which are often resistant to all other clinically approved drugs. Vancomycin resistance in enterococci is often plasmid mediated and can be the result of several unique resistance determinants.[99,100] Guidelines from the Hospital Infection Control Practices Advisory Committee of the Centers for Disease Control and Prevention (CDC) and the Society for Healthcare Epidemiology of America[98,101] stress the need for prudent use of antimicrobial agents, effective implementation of infection control in health-care settings, and the requirements for adequate laboratory support of infection control. All are of critical importance to reduce the selective pressure for the emergence of resistant microorganisms. Antibiotic use, particularly of vancomycin and cephalosporins, is frequently a risk factor for developing VRE infections.[102] Programs to promote proper stewardship of antimicrobial use have become frequent in health-care organizations, and guidelines for such programs are being developed by professional societies.[54]

Several species of multiresistant gram-negative organisms also continue to be significant pathogens in hospitals.[103] For example, Enterobacteriaceae resistant to aminoglycosides, β-lactams, chloramphenicol, and trimethoprim–sulfamethoxazole emerged in the late 1970s and have become endemic in a number of hospitals in the

United States.[9] Many of these organisms now manifest resistance to the extended-spectrum cephalosporins as well. However, while strains of *K. pneumoniae* primarily are resistant due to the production of ESBLs, *Enterobacter* species are more likely to have chromosomally encoded enzymes related to the AmpC family of β-lactamases.[35] Transmissible imipenem resistance was noted in *P. aeruginosa* in the late 1980s, and now has become a major concern in several parts of the world.[13] *Acinetobacter* species, especially *A. baumannii*, have caused outbreaks in intensive care settings in selected hospitals,[104] and have been associated with infections in military personnel returning to the United States from the Middle East.[105]

5.2. Community-Acquired Pathogens

Development and spread of resistance in community-acquired bacteria have compromised many established therapeutic regimens for infectious diseases.[17,106] The increase in resistance has resulted from increased world travel where people are exposed to novel microorganisms that they bring back to the home country; greater reliance on foodstuffs, such as fruits and vegetables, from international sources that may be contaminated; and dramatic increases in worldwide antibiotic use. For example, in 2004, outbreaks of dysentery in India were reported that were caused by *Shigella dysentariae* strains resistant to all major drug classes, including the quinolones.[107] These organisms contained a broad array of resistance genes, apparently assembled from a variety of enteric bacteria. Multi-drug resistance in *Salmonella typhi*, the cause of typhoid fever, has also been noted, with decreased susceptibility to the fluoroquinolones, which will further complicate the treatment of typhoid fever because few options are available.[108] Widespread use of fluoroquinolones in hospitals for staphylococcal infections in the early 1990s led to the rapid and dramatic increase in resistance,[83] and this class of drugs has significantly reduced effectiveness in many hospitals for treating staphylococcal infections.[109] The emergence of methicillin-resistant staphylococcal strains centered in the community with different characteristics than those previously encountered were dubbed community-acquired MRSA (CA-MRSA). Such strains now are spreading into health-care settings as well; this complicates treatment and prevention strategies for this common cause of community-acquired skin and soft tissue infections.[15,110] Gram-negative bacilli that are part of endogenous flora, such as *E. coli* and *K. pneumoniae*, are also associated with increasing prevalence of resistance to antimicrobial agents commonly used in community-acquired infections.[111,112] The impact is especially notable in treatment for community-acquired urinary tract infections in women.[113]

Another important community-acquired pathogen, *S. pneumoniae*, is a major cause of meningitis, sepsis, pneumonia, and ear infections in the United States and around the globe.[114] Strains resistant to penicillin, tetracycline, chloramphenicol, and trimethoprim–sulfamethoxazole have now been isolated in the United States[115] and in several areas of the world.[116] In the Atlanta area in 1994, 25% of pneumococcal isolates were no longer susceptible to penicillin and required alternate drugs for therapy of serious infections.[117] Other strains of pneumococci highly resistant to the extended-spectrum cephalosporins, the drugs of choice for treatment of penicillin-resistant strains, have emerged and appear to be spreading.[118] This has significantly complicated the development of rational plans for treatment of meningitis caused by these strains, and forced inclusion of vancomycin in initial empiric treatment regimens.[119] Pneumococci can spread particularly well among children in day care centers, but pediatric strains of pneumococci are also now found with increasing frequency in adults.[120] Thus, the availability of more effective pneumococcal vaccines that are immunogenic in younger children (especially those $\leq$2 years of age) may play a significant role in controlling the spread of these resistant pathogens.[121,122]

Another community-acquired pathogen that has engendered considerable interest due to its ability to acquire multiresistance is *Neisseria gonorrhoeae*, a sexually transmitted agent that has been epidemic in the world for almost three decades. High-level plasmid-mediated penicillin resistance emerged in *N. gonorrhoeae* in 1976.[123] The β-lactamase gene was similar to one found just 1 year previously in strains of *Haemophilus influenzae*.[124] Today, quinolones are key therapeutics for gonorrhea, and the emergence of resistance to this group of drugs threatens once again our ability to control this epidemic disease.[125]

Community-acquired infections due to *Neisseria meningitidis* continue to occur in epidemic fashion in developing countries and in localized outbreaks in developed countries. A key part of response to the severe disease produced by this organism is vaccination.[126]

6. Unresolved Problems

The unresolved problems of the resistance story are threefold: How to identify new targets for antimicrobial action for which resistance will be slow to develop; how to eliminate the spread of resistance genes located on plasmids and transposons to other organisms; and, most importantly, how to reduce the selective pressure in community and

hospital settings that encourage the development and spread of antimicrobial-resistant microorganisms. Attention to each of these problems will help lessen the impact of antimicrobial resistance on human and animal health and prolong the effectiveness of the antimicrobial agents that we do have.

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CHAPTER 5

Anthrax

Philip S. Brachman and Arnold F. Kaufmann

1. Introduction

Anthrax, a zoonotic disease of herbivorous animals transmissible from animals to man, occurs primarily in three forms: cutaneous, inhalational, and gastrointestinal. Meningitis and septicemia occur but are secondary to one of the primary forms; occasionally, cases of anthrax meningitis are reported in which a primary focus is not identified. The etiologic agent is *Bacillus anthracis*, a gram-positive organism that in its spore form can persist in nature for prolonged periods, possibly years. The incidence of anthrax has decreased over the past 70 years, so that currently, human cases are seen only occasionally in the United States, where reporting is probably fairly accurate. Synonyms for anthrax include charbon, malignant pustule, Siberian ulcer, malignant edema, woolsorter's disease, and ragpicker's disease.

2. Historical Background

The earliest description of anthrax is found in the book of Genesis, the fifth plague (1491 BC), which appears to have been anthrax, was described as killing the Egyptians' cattle. There are descriptions of anthrax involving both animals and humans in the early literature of the Hindus and Greeks. Virgil described anthrax during the Roman Empire (70–19 BC). In the 17th century, a pandemic referred to as "the black bane" swept through Europe, causing approximately 60,000 human deaths and many animal deaths. An association between human anthrax and contact with clothing made from wool and hides was reported.

In 1752, Moret more specifically characterized the disease in man, calling it "the malignant pustle." In 1780, Chabert described the disease in animals. The contagious nature of anthrax was noted in 1823 by Barthelemy. The first microscopic description of the organism was written by Delafond in 1838, and the organism was first described in infected animals in 1849 by Pollender. Pasteur discussed anthrax in some of his earlier writings on his germ theory of disease. In 1876, Koch used *B. anthracis* in developing his postulates concerning the relationship between bacteria and the diseases they cause. Greenfield developed an attenuated animal spore vaccine in 1880.[1–3] Pasteur, responding to a serious problem with anthrax in the French livestock industry, developed and field tested in sheep an attenuated spore vaccine in 1881. In 1939, Sterne reported his development of an animal vaccine that is a spore suspension of an avirulent, noncapsulated live strain.[4] This is the animal vaccine currently recommended for use.

Anthrax in the United States was first reported among animals in Louisiana in the early 1700s. Subsequently, sporadic animal cases have been reported from throughout the United States, and epizootics have been reported from the southern portion of the Great Plains states and the northeastern part of the country. The first human case was reported in a cattle tender in Kentucky in 1824.

Occupational anthrax occurred in the mid-1800s in England, where it was known as woolsorter's disease,[1] and in Germany, where it was known as ragpicker's disease. Ragpicker's disease occurred in individuals who handled rags that had been woven from contaminated animal fibers. The increasing problem of woolsorter's disease in England led to the development of a government inquiry committee that correctly identified the problem as being related to imported animal fibers contaminated with *B. anthracis*.[5] Subsequently, in 1921, a formaldehyde disinfecting station was built by the government in Liverpool.[6] All "dangerous" imported

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wools and goat hairs were first washed in formaldehyde baths, which successfully reduced contamination of the animal fibers with *B. anthracis*. The occurrence of inhalational anthrax was drastically reduced, so that only sporadic cases have been reported in England since 1922. The disinfecting station continued to such a level that further disinfection with formaldehyde was not deemed necessary or economical.

In the United States, human anthrax cases have been reported from most of the states. Initially, cases were related to animal contact and reflected areas with enzootic and epizootic anthrax, primarily involving cattle and occasionally sheep and horses. As the United States became industrialized, human cases associated with the textile and tanning industries occurred with increasing frequency. In the early 1900s, these cases primarily occurred in the northeastern states. In the 1950s, cases began to occur in southeastern states, reflecting the movement of the industry.

In the 1920s, the annual number of cases reported in the United States ranged from 100 to 200 cases; in the 1950s, from 20 to 50 cases each year; in the 1970s, from 0 to 6 cases each year; in the 1980s and in the 1990s, one case was reported; from 2000 to 2008, 28 cases were reported that includes the 22 cases that resulted from a bioterrorism event associated with the purposeful contamination of mailed letters and subsequent contact with postal facilities or contaminated letters. One subsequent case occurred in a laboratory employee who was working with *B. anthracis* associated with the bioterrorist event. The decrease in cases (excluding the bioterrorist cases) is the result of the use of a human cell-free anthrax vaccine among high-risk industrial groups, decreased utilization of imported potentially contaminated animal products, improved hygiene in industry, and improved animal husbandry. The incidence of animal anthrax has likewise decreased so that only occasional cases are reported and epizootics have not been reported for a number of years. This reduction is the result of improved animal husbandry and the appropriate utilization of animal anthrax vaccine.

3. Methodology

3.1. Sources of Mortality and Morbidity Data

In the United States, data on human anthrax are collected in individual states through the routine surveillance system. Usually, individual case data are obtained by the use of special case-reporting forms and investigations. These data are then reported by means of the morbidity reporting system (National Notifiable Diseases Surveillance System—NNDDS) to the Centers for Disease Control and Prevention (CDC). Epidemic information and, at times, individual case data are reported to the CDC by telephone. Years ago, the reporting of anthrax was spotty; however, as health-care providers have accepted their responsibilities to notify public health officials about the occurrence of reportable disease, overall surveillance has improved. Additionally, as the incidence of the disease has decreased and the use of laboratory in assisting in diagnosing the disease has increased, reporting has become more complete.

Due to increasing concern about bioterrorism, potential bioterrorist agents have been classified according to their significance as agents. Category A agents are those with the greatest potential for causing the most significant morbidity and mortality and *B. anthracis* is in this category. The isolation and identification of any of these agents are to be reported immediately by telephone to CDC.

Data concerning anthrax in animals are obtained through federal and state departments of agriculture or public health or both.

Worldwide data on anthrax are available from the World Health Organization (WHO) and the Food and Agriculture Organization. However, not all countries maintain adequate surveillance programs for infectious diseases, and thus all do not have data available on cases of human or animal anthrax. Reports of anthrax associated with rural areas, i.e., animal-associated cases, will be especially deficient because of the generally poorer reporting of diseases from rural areas. Another source of data is ProMed, the worldwide computerized program for the reporting of the occurrences of infectious diseases in which a report of a case or an epidemic of anthrax may be published, even though it was not officially reported to the country's central health authority. Additionally, reports of epidemics in the scientific literature may give the actual number of cases of the disease that occurred, whereas the number if reported to the health authorities may only include those cases confirmed in the laboratory.

3.2. Surveys

Population surveys are not practical due to the low incidence of the disease. Serological surveys are not generally useful for obtaining information on anthrax, except in epidemic or epizootic investigations, because of limitations of the laboratory procedures. However, they may also be useful in ascertaining immunity levels in human and livestock populations.

3.3. Laboratory Diagnosis

3.3.1. Isolation and Identification Swabs from cutaneous lesions, vesicular fluid, or blood may be examined for

the presence of *B. anthracis*.[7] *Bacillus anthracis* is a gram-positive, nonmotile, hemolytic, spore-forming bacillus (1–1.3 ×.3–10 μm) that grows on ordinary laboratory media at 37ºC, producing round, grayish-white, convex colonies having comma-shaped outshootings with a ground-glass appearance that measure 2–5 mm in diameter. Colonies first appear approximately 8–12 h after inoculation of the agar and show the typical characteristics 24–36 h after inoculation. When an inoculated loop is drawn through the colony, tenacity is demonstrated; the disturbed part of the colony will be drawn perpendicular to the agar and will remain in this position, resembling beaten egg white. Microscopic examination (such as by Gram's stain) of growth from artificial media shows long parallel chains of organisms with square ends, described as "boxcars." Spore stains will reveal central or paracentral spores in *B. anthracis* organisms that have incubated at least 24 h. Material from a fresh lesion will show shorter chains with single or two to four organisms in a row with slightly rounded ends. Direct fluorescent-antibody staining specific to either the cell wall or the capsule may also be used to identify the organisms from vesicular fluid cultures or from tissues.[8] A newer method to identify *B. anthracis* in formalin-fixed tissue sections is immunohistochemical staining using monoclonal antibodies against *B. anthracis* cell wall and capsule.[9]

A bacteriophage designated gamma phage may be used to confirm the identification of *B. anthracis*.[10] Toxin production assays and DNA probes (PCR) for the presence of pX01 and pX02 plasmids are newer identification methods available in some reference laboratories.[11] The DNA probe can be sued to identify *B. anthracis* in nonviable specimens. Subcutaneous or intraperitoneal inoculation of guinea pigs, mice, or rabbits with agar-grown cells suspended in saline may be useful in differentiating *B. anthracis* from other gram-positive bacilli. *Bacillus anthracis* will cause death from 24 to 72 h after inoculation, and the animal will show evidence of general toxicity and multiple organ hemorrhages. Animals inoculated subcutaneously demonstrate subcutaneous gelatinous, hemorrhagic edema in addition to general toxemia. Broth-grown cultures must not be used because nonspecific death can result.

Tissue from autopsied patients should be cultured and microscopically examined.

3.3.2. Serological and Immunologic Diagnostic Methods. The virulence of *B. anthracis* is determined by the presence of three components: edema toxin, lethal toxin, and capsular material. To exert their effect within cells, both toxins require participation of a common transport protein called protective antigen. The capsule material contains poly-D-glutamic acid, which helps protect the bacillus from ingestion by phagocytes. Production of the toxic factors is regulated by one plasmid (pX01) and that of the capsular material by a second plasmid (pX02).

An enzyme-linked immunosorbent assay (ELISA) has been developed to measure antibodies to the lethal and edema factors and the protective antigen. A fourfold rise in titer or a single titer of greater than 1:32 is indicative of current or recent infection or acquired immunity.[12,13] An electrophoretic-immunotransblot test (western blot) that measures antibody to the protective antigen and the lethal factor is used to confirm ELISA results, or as the primary diagnostic test. Assays have been developed to detect the presence of the protective antigen component of the toxin in vesicular fluid as well as serum and cerebrospinal fluid (CSF).[12,14] There is a rapid card test that is used in veterinary medicine.[14]

3.3.3. Pathology. Autopsy material, commonly from the mediastinal structures, lymph nodes, spleen, and liver, should be examined for organisms and pathological changes.[15] The most significant findings at autopsy are those seen in patients who have died of inhalational anthrax. The classic finding is that of hemorrhagic mediastinitis with enlarged, hemorrhagic lymphadenitis. There may be inflammation of the pleura and some pleural effusion. Acute splenic may also be seen. Some patients may have hemorrhagic meningitis, and in one patient, hemorrhages were seen in the gastrointestinal tract.[16]

In deaths due to the abdominal form of gastrointestinal anthrax, there typically is hemorrhagic enteritis with congestion, thickening, and edema of the intestinal walls. Mucosal ulcers with necrosis may be seen in the stomach, terminal ileum, and cecum. The regional lymph nodes are enlarged, edematous, and hemorrhagic with some necrosis. There may be acute splenitis. Peritonitis with ascetic fluid is present.

In some patients with significant cutaneous lesions on the head and neck or the oropharyngeal form of gastrointestinal anthrax, marked anterior cervical subcutaneous edema may compress the trachea at the thoracic inlet. Death in such patients is due to asphyxiation.

4. Biological Characteristics of the Organism

The resistance of the spore form of *B. anthracis* to physical and chemical agents is reflected in the persistence of the organism in the inanimate environment. Organisms have been demonstrated to persist for years in buildings formerly used as factories in which the environment became contaminated during the processing of contaminated imported materials of animal origin. Accordingly, they may serve as the source of infection for people who work in the area. Special efforts are required to decontaminate this

environment; one method is to use paraformaldehyde vapor, which is successful in killing *B. anthracis* spores. However, due to concern about the potential carcinogenicity formaldehyde products, other disinfectants have been substituted such as chlorine dioxide and hydrogen peroxide vapor.[17] In the mail-associated bioterroristic event of 2001, the internal environment of post offices, commercial buildings, and commercial offices became contaminated with *B. anthracis*. Decontamination of large buildings is expensive, with the use of a protocol primarily dependent on aqueous chlorine at one mail handling facility costing $464,000. The cost of decontaminating the office building on Capitol Hill was approximately $28 million and the cost for decontaminating the two large mail centers was approximately $200 million.[17] In the laboratory, surfaces may be decontaminated with either 5% hypochlorite or 5% phenol (carbolic acid); instruments and other equipment may be autoclaved.

Bacillus anthracis may also persist in certain types of soil for years.[18] Alluvial soil with a pH greater than 6.0 is best suited for survival of *B. anthracis*. Persistence of the organism in soil plus environmental conditions such as climatic variations, i.e., flooding or drought, triggering brief periods of bacterial growth thought to be associated with outbreaks of anthrax in animal anthrax are known as anthrax districts. One such area is the lower Mississippi River valley area, from which sporadic outbreaks of animal anthrax have been reported at irregular intervals for years. The molecular structure of *B. anthracis* organisms can be typed and this has proven useful in defining the geographic distribution of specific strains.[19] The same technology has been used in forensic investigations.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

In 1958, Glassman,[20] using reports from the WHO, estimated the annual worldwide incidence of human anthrax to be from 20,000 to 100,000 cases. Data from the WHO[21] for 1981 reported 3,200 cases from 43 countries, which undoubtedly is an underestimate. A review of reports from the WHO, Food and Agricultural Organization, and the Office Internationale des Epizooties for the 1900s provides some more recent data, but it is no more accurate than the previous estimates. In 1993, 1915 human cases were reported from 16 countries with 14 other countries reporting no cases, and in 1994, 694 cases were reported from 13 countries with another 17 countries reporting no cases. Animal anthrax remains an endemic problem in various Asiatic, African, South American, and Caribbean countries, as well as in Australia.[22] In countries with a significant animal anthrax problem, there is probably a related human anthrax problem. In China, for example, an average of 2,115 human cases were reported annually in the period 1989–1993, primarily related to butchering and consuming infected livestock.[22] However, since the reporting of diseases from rural areas is sporadic, these data are not generally available. Industrial anthrax infections are related to the processing of animal products such as goat hair, wool, skins and hides, and dried bones. Industrial countries that process these materials will have industrially related cases at a rate inversely proportional to the level of development of appropriate hygienic measures directed at reducing the threat of anthrax.

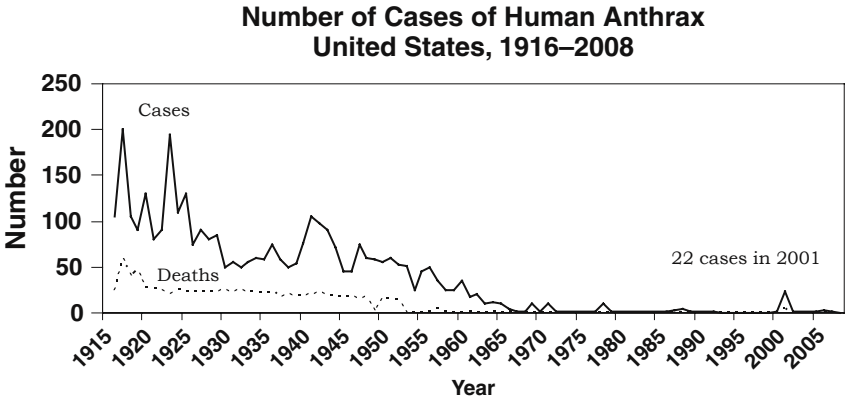


Figure 1. Anthrax in man in the United States, 1916–1996.
Source: NOVS and MMWR data.

In the United States, the annual number of cases of human anthrax decreased from an average of 127 for the years 1916–1925 to 44 cases for 1948–1957, to 9.6 cases for 1958–1967, to 2.4 cases for 1968–1977, to 0.9 case for 1978–1987, to 0.3 case for 1988–1996, to 0.45 cases (excluding the bioterrorist cases) or 2.55 cases (including the bioterrorist cases) for 1997–2007 (see Figure 1).

Approximately 95% of anthrax cases in the United States are cutaneous and 5% are inhalational; confirmed cases of gastrointestinal anthrax have not been reported in the United States but have been reported in other countries. Some investigators report that from 5 to 10% of cases worldwide may be gastrointestinal cases, i.e., one such case to every 100–200 cutaneous cases. From 1955 to 1996, 262 cases of anthrax were reported in the United States; 240 cases were cutaneous (21 of which were at unknown sites) and 22 were inhalational. Of the 203 cutaneous cases for which the sites of infection were reported, the majority occurred on the exposed part of the body, with 51% on the arms, 27% on the head and neck, 5% on the trunk, and 3% on the legs (see Table 1).

It is estimated that approximately 20% of untreated cases of cutaneous anthrax will result in death. Of the 224 cutaneous cases that have been reported in the United States since 1955, 11 have been fatal. Prior to 2001, inhalational anthrax was almost always fatal; between 1900 and 2000, 16 of the 18 cases reported in the United States were fatal.[23] However, in the bioterroristic event of 2001, six of the 11 inhalation cases survived due to rapid and aggressive treatment (see Section 8.1.2).[23]

In the few reports available, the mortality from gastrointestinal anthrax has usually ranged from 25 to 60%. In 1989, a gastrointestinal anthrax epidemic in Tibet resulted in 507 cases, including 162 deaths (32%).[24]

Table 1. Sites of 264 Anthrax Infections, United States, 1955–2007

Sites	Infections	Distribution	
		Number	Percent
Cutaneous	220		
Arms		131	50
Head and neck		69	26
Trunk		12	5
Legs		8	3
Unknown	21	21	8
Inhalation	23	23	9

5.2. Epidemic Behavior and Contagiousness

The first reports of inhalational anthrax (woolsorter's disease) in England in the late 1800s and early 1900s included clusters of cases associated with the sorting of certain lots of imported wool, frequently mohair.[25] In the United States, occasional epidemics occurred in industrial settings, epidemiologically related to the processing of batches of highly contaminated imported animal fibers, particularly goat hair. These epidemics were primarily of cutaneous anthrax.

The largest epidemic prior to 2001 reported in the United States occurred in 1957, and involved employees in a goat-hair-processing plant.[26] Among the 600 employees over a 10-week period, there were nine cases of anthrax: four cases of cutaneous anthrax and five of inhalational anthrax. None of the patients with cutaneous anthrax died, but four of the five with inhalational anthrax died. Epidemiological investigations related the cases to a particular contaminated batch of goat hair imported from Pakistan.[27] More than 50% of samples from this particular batch were culture positive for *B. anthracis*. A detailed examination of production records revealed that each of the patients could have had direct contact with this particular lot of goat hair during an appropriate period before the onset of disease.

An outbreak of human anthrax occurred in north central Russia (Sverdlovsk) in 1979, in which the source of infection was an accidental release of *B. anthracis* aerosol from a biological warfare research laboratory.[28] The exact number of cases is uncertain, but at least 77 persons developed inhalational anthrax subsequent to exposure in a narrow 4-km-long zone south of the laboratory. Deaths in sheep occurred up to 50 km from the laboratory as a result of the same accidental release. However, anthrax in sheep had been reported for years before the accident in Sverdlovsk, so whether these cases are related to the accidental release or not is not clear. This incident is the largest inhalational anthrax epidemic reported.

Over a 2-month period, September to early November, 2001, 22 cases of anthrax, 11 inhalational and 11 cutaneous, occurred in Florida, New York, New Jersey, Connecticut, and in Washington, DC. The source of infection was primary- and secondary-contaminated mail and the subsequent contamination of the environment of involved post offices and office buildings. Four recovered mailed letters contained powder and *B. anthracis* spores; there were more such letters, but they were not recovered. It is theorized, with good epidemiologic supporting evidence, that other letters being processed in post offices could have been secondarily contaminated with *B. anthracis* spores that then served as the source of infection for others.[29,30]

Human-to-human transmission of anthrax has rarely been reported. A recent report on anthrax in China included information on 10 cases of person-to-person transmission in a medical staff.[24] However, data are not presented that confirm these cases as representing human-to-human transmission. Industrial infections result from exposure to the organisms in the industrial environment, from contact with organisms either in the materials actually being processed, or in the environment that has been contaminated by previously processed materials. Those employees who work in the earliest processing stages, in which the materials and the environment are more heavily contaminated with *B. anthracis*, are at greater risk of contracting anthrax than those who work with the materials at later stages in the manufacturing cycle. Occasional cases occur in persons who have direct or indirect contact with *B. anthracis* in a laboratory setting.

Agricultural cases occur in veterinarians and others who have direct contact with animals that have died of anthrax. There have been cases of human disease associated with epizootics of anthrax in cattle. The largest reported agricultural outbreak occurred in Zimbabwe with more than 10,000 cases reported between 1979 and 1985.[31] The peak number of cases, more than 6,000, were reported to have occurred in the involved area. The majority of patients had cutaneous infections located primarily on the exposed part of the body; some gastrointestinal cases were reported. Domestic cattle deaths were also noted. The humans were infected from contact with the diseased animals or by handling their carcasses during butchering or burial; the source of infection for the animals was not reported. Routine animal vaccination had not been practiced.

5.3. Geographic Distribution

Cases are distributed according to their association with an agricultural or industrial source of infection. From 1920 to 1954, more than 50% of all cases in the United States occurred in Pennsylvania, New York, New Jersey, Massachusetts, and New Hampshire, the location of industries that processed animal hair, hide, and bone.[32] Subsequently, industrial cases began to be reported from other states, such as North Carolina and South Carolina, as companies moved southward. In more recent years, cases have not shown a geographic localization, reflecting the decrease in industry-related cases and the low endemicity of agriculture-related cases.

Agriculture cases are distributed in rural areas associated with outbreaks in animals and primarily occur in the previously discussed anthrax districts. Some of these districts are located in Louisiana, Mississippi, and South Dakota.[18] Domestic epizootics commonly involve cattle, but sheep, swine, and horses are also susceptible. Cases have also been reported involving white tailed deer and moose. In Canada, cases have been reported in buffalo. Cases and epizootics have also been reported in domestic and wild animals in Asia, Africa, Europe, especially eastern Europe, and Australia. The source of infection for wild animals in captivity is infected foods; for domestic range cattle or wild animals not in captivity, the source is contaminated soil. Biting flies have been reported as transmitters of infections.[33]

5.4. Temporal Distribution

Industrial cases occur throughout the year without any seasonal pattern. Animal-related cases occur primarily in the spring and summer, the seasons when animal anthrax cases occur with the greatest frequency.

5.5. Age

The age distribution of cases reflects the age of individuals who work in the involved industry or come in contact with infected carcasses. Primarily, these persons are 20–60 years of age. Occasional cases have been reported in children, one of whose parents worked in a factory in which *B. anthracis*-contaminated materials were processed; it has been hypothesized that *B. anthracis* organisms were transported into the home on the parent's clothing. In Haiti, the distribution of cutaneous anthrax cases by age group was reported as being generally comparable to that of the general population, with the anatomic distribution of lesions being similar in all age groups.[34]

5.6. Sex

The predominance of males reflects the sex distribution of the people who work in the involved industries or have contact with infected carcasses.

5.7. Race

No distinctive racial patterns are found among persons with anthrax.

5.8. Occupation

Anthrax cases may be divided into two major categories: those associated with an industrial setting and those associated with an agricultural setting (see Table 2). However, some cases occur in people who live in urban areas in which the source of infection is unknown; in these cases, the source is assumed to be industrial. In addition, a few laboratory-associated cases have been reported.

Table 2. Source of Infection in 264 Cases of Human Anthrax, United States, 1955–2007

Source	Number
Industrial	
Goat hair	113
Wool	34
Goat skins	19
Meat	3
Bone	4
Unknown	12
Agricultural	
Animal	46
Vaccine (animal)	2
Unknown	8
Bioterrorism	
Direct	22
Indirect (laboratory)	1

Industrial cases have occurred in individuals who worked in industries in which animal products, usually imported, were processed.[32,35] In the United States, this primarily involved workers in goat-hair- and wool-processing industries. Goat hair was imported primarily from Asiatic, Middle Eastern, and African countries in bales weighing 200–250 lb each. The hair was processed into yarn used in preparing interlinings for men's suit coats and also into a felt material used as underpads for carpets, insulation for pipes, saddle pads, and washers. The wools that have been implicated in cases of anthrax are imported from the same areas of the world and are primarily the coarser wools used in carpets as distinct from the finer wools used in clothing. Occasionally, however, anthrax has been associated with fine cashmere wool.

Anthrax has also been associated with the tanning industry and has resulted from contact with imported skins and hides. Cases have also been associated with the bone-meal-processing industry, in which dried bones are imported for preparing bone meal for fertilizer, animal food supplements, or gelatin. The dried bones are usually gathered from animal grazing areas and animals that have died of unknown causes. However, bones may also be obtained from slaughtering or rendering plants.

There have been cases in which the source of infection has been domestic animal products. For example, in 1924, a number of persons developed cutaneous anthrax following their contact with hides from animals that died during an epizootic of anthrax.

Occasional cases have been reported among persons who have had contact with horsehair and pig bristles that are used in brushes, such as shaving brushes or hair brushes. The number of cases associated with contaminated imported shaving brushes was significantly reduced in the 1920s by the enactment of laws requiring that these brushes be shown by culture to be free of B. anthrax organisms when imported into the country. In recent years, the use of animal fibers has decreased as synthetic materials have been used in increasing quantities. Accordingly, this risk of anthrax is negligible. Among the 22 cases of anthrax associated with bioterrorists event of 2001, 12 were mail handlers, nine occurred in persons who were United States Postal Service employees.[29]

Agricultural cases have also occurred in individuals who used fertilizer or animal feed that contained contaminated bone meal. One particular epidemic related to feed supplement for pigs involved 846 cases in pigs, with some cases in persons who had contact with the pigs.[36]

Occasional cases have occurred in individuals who have had no known contact with industrial or agricultural sources. Usually, their cases are thought to have been caused by environmental contamination such as in the case of a housewife who lived near a tanner and died of inhalational anthrax. Another case occurred in an individual who every day walked by the receiving room door of a tannery where contaminated hides were processed.[37] It was shown that air could have flowed from the receiving area out into the street, where the individual could have inhaled an infecting dose of airborne *B. anthracis* organisms. At least 77 cases of inhalational anthrax occurred in Sverdlovsk, Russia in 1979, associated with the accidental release of an aerosol from a biological warfare research laboratory.[28]

Several cases have occurred in persons whose contact has been in laboratories in which strains of *B. anthracis* have been handled.[38] Two of these cases were fatal inhalational cases.

5.9. Cases Related to Commercial Products

An occasional case has occurred in an individual after contact with a commercial product prepared from animal materials. Examples of cutaneous anthrax include contact with imported souvenir drums with drumheads made of goatskin[39] and a case in which the source of infection was most likely a newly purchased woolen coat. A fatal case of inhalational anthrax occurred in a home weaver following contact with imported yarn that contained animal fibers.[40] Three cases of anthrax in the United States, one inhalational, occurred in individuals who worked with imported animal skins that were being processed into drum heads.[41,42]

5.10. Socioeconomic and Other Factors

Socioeconomic or other factors may contribute significantly to the risk of an anthrax infection. Among some people in certain societies, economic problems lead to individuals salvaging the meat, hair, or hides from carcasses of

animals that died from gastrointestinal anthrax and related to butchering and consuming raw or undercooked meat from anthrax-infected carcasses, they developed anthrax.

6. Mechanisms and Routes of Transmission

In the United States, *B. anthracis* organisms are primarily transported by the contact or the airborne routes. Organisms can also be transmitted by a common vehicle, food (meat), though this is rare. Vectors (flies) have been reported to transmit organisms, although the actual relationship to subsequent disease has not been proven. In Asian and African countries, the gastrointestinal route is more common.

Contact spread is primarily by indirect contact, although direct contact and droplets may also be routes of transmission, and results in cutaneous lesions, most frequently on the exposed parts of the body. Direct contact spread occurs when a susceptible host has direct contact with an animal that is infected with *B. anthracis*, such as a veterinarian who autopsies an animal contaminated with the organism. Organisms may enter the body through a preexisting skin wound or may be accidentally injected by a sharp bone spicule or a knife.

Meat from an animal that died from anthrax is usually infected and can serve as a source of infection if eaten undercooked. Persons preparing the meat for consumption are at risk of cutaneous anthrax.

Indirect contact spread accounts for the majority of cutaneous industrial cases. The source of infection is either the animal products being processed or the environment that has been contaminated by animal products previously processed. Rarely, the finished product may be the source. By this route, organisms enter the skin through a preexisting wound, a new wound, or the mucous membranes (i.e., conjunctivae). Goat hair fibers may be flung from the manufacturing equipment like projectiles and enter the skin like needles, depositing *B. anthracis* organisms in the subcutaneous tissues.

Aerosols containing *B. anthracis* spores may be created by agitation of the hair or wool either when the bales are being opened or the fibers initially handled or as the fibers are being processed through the machinery. These aerosols may travel more than several feet, which represents airborne transmission. Particles less than 5 μm in diameter, if inhaled, may reach the terminal alveoli of the lungs and cause inhalational anthrax (see Section 7.1.2). The estimated human LD_{50} dose from inhaled small-particle aerosols has been estimated to be 8,000–10,000 spores.[43] The minimum infecting dose for humans is unknown. Essentially all the risk of infection occurs during exposure to the primary aerosol generated during processing. Inefficiency of small-particle aerosol deposition and resuspension minimizes any potential risk of significant secondary aerosol.[28]

The air in several goat-hair-processing plants has been sampled, and significant levels of airborne *B. anthracis* spores have been identified. For example, studies in a goat-hair-processing plant in the Northeast in 1958 demonstrated that "510 spores in particles 5 microns and less in size may be inhaled [by workers] in 8 hours without inducing infection."[43] In a similar mill in a southern state, 91 primates (cynomolgus monkeys) were exposed during a series of experiments to the air in the dustiest part of the mill.[44] They had an anthrax mortality rate of 10–25% from a calculated inhalation dose of 1,000–5,000 *B. anthracis* organisms accumulated over 3–5 days.

Animals become infected by ingestion of contaminated soil or feed. Some rural areas are associated with sporadic cases or outbreaks of animal anthrax. The soils in these areas are most frequently in the neutral pH range and are alluvial or loessal soils. Soil becomes contaminated from the discharges or carcasses of dead animals. Soil can also become contaminated from use of contaminated fertilizer or feed. It is reported that the spores may remain infectious in nature for many years, though this has never been scientifically proven. There is evidence to suggest that in some areas *B. anthracis* organisms can be considered as part of the endogenous soil flora. With certain climatic conditions, the spores germinate and multiply, resulting in an increase in the number of infectious organisms that could be ingested by susceptible grazing animals.

Insect vectors, such as horseflies, have been reported to transmit *B. anthracis* by means of mechanical transfer of *B. anthracis* organisms from contaminated foci to individuals or animals.[33] Outbreaks of animal anthrax have been reported to result from transmission of *B. anthracis* by horseflies from an infected animal to a second animal. However, there is no scientific documentation, including field studies during an epizootic, that this potential method of transmission has any practical significance.

7. Pathogenesis and Immunity

7.1. Pathogenesis

7.1.1. Cutaneous Anthrax. The incubation period is from 3 to 10 days, most commonly 5–7 days. However, there have been some reports of lesions developing as soon as 9 h after the reported exposure.[45] Spores deposited beneath the skin germinate, and the resulting vegetative forms multiply and produce a toxin. The local lesion results from the action of the toxin on the surrounding tissue, which causes tissue

necrosis. This leads to the development of a scar at the site of the lesion. The toxin or organisms or both may be distributed throughout the body by the vascular system, causing systemic symptoms and signs of toxicity or bacteremia. Occasionally, organisms are picked up by the lymphatic system, resulting in lymphangitis and lymphadenopathy.

7.1.2. Inhalational Anthrax. The incubation period is usually stated to be 1–5 days (mean 3–4 days). However, in the epidemic of inhalational anthrax in Sverdlovsk, the incubation periods ranged from 4 to about 45 days (median 9–10 days).[28] The explanation for the prolonged incubation period is unknown, but may represent low-dose phenomenon or prophylactic use of antibiotics. *Bacillus anthracis*-bearing particles less than 5 μm in size are inhaled and, if they reach the terminal alveoli, are deposited on the alveolar membranes, where they can be ingested by alveolar macrophages, carried across the membranes to the regional lymph nodes, and deposited. Spores will then germinate, multiply, and produce toxin. The toxin, in turn, destroys tissue and subsequently causes necrosis and hemorrhage. The classic picture at death is very distinctive. Examination of the mediastinal area reveals hemorrhagic mediastinitis with varied degrees of destruction of the normal architecture.[46,47] While the pathological changes are limited to the mediastinal area, there may be secondary involvement of the pulmonary tissue. Toxin may be distributed throughout the body, resulting in systemic symptoms and signs. Organisms may also be picked up by the vascular system, resulting in bacteremia and septicemia. The pathogenesis and radiological findings of inhalational anthrax can be found at http://anthrax.radpath.org.

7.1.3. Gastrointestinal Anthrax. The incubation period is commonly 3–7 days. There are two clinical presentations following ingestion of *B. anthracis*-contaminated food: abdominal and oropharyngeal.

In the abdominal form, spores are ingested, absorbed through the intestinal mucosa, probably via M cells in the epithelium overlying Peyer's patches, and deposited in mucosal lymphoid tissue or regional lymph nodes; here, they germinate, and the vegetative cells multiply, producing toxin. An ulcerative lesion caused by the action of the toxin may develop within the mucosa of the gastrointestinal tract. The lesions are frequently described in the cecum and adjacent areas of the bowel. Some reports have described lesions in the large bowel, stomach, and rarely in the duodenum.[48] Alternatively, lymphadenitis or lymphadenopathy may occur. Hemorrhagic areas may develop within the mesentery as well as in the gastrointestinal tract.

In the oropharyngeal form, organisms are transported through the oral mucosa to the tonsils or cervical lymph nodes, where they germinate, multiply, and produce toxin. The result may be edema and tissue necrosis in the cervical area. There is a report from Thailand as well as several other countries of the development of an inflammatory lesion resembling a cutaneous lesion in the oral cavity involving the posterior wall, the hard palate, tongue, or the tonsils.[49]

7.2. Immunity

Apparently, once having had a cutaneous lesion, an individual develops some degree of immunity against another infection. There have been no well-confirmed second cases of cutaneous anthrax in individuals who have had a confirmed first case. There have been several reports of two cutaneous anthrax infections in the same individual; however, in each of these reports, either one or both of the cases of anthrax have not been well documented.[45]

Extensive serological studies have not been conducted. In one study, however, Norman et al.[50] studied 72 unvaccinated employees in a goat-hair-processing mill, none of whom had a history of an anthrax infection. Of the 72, 11 demonstrated a positive titer (precipitation-inhibition test), suggesting previous subclinical infection. Recovering cutaneous and gastrointestinal anthrax patients develop antibody to the toxin components as determined by ELISA and electrophoretic-immunotransblot (Western Blot) testing.[51]Antibody and cell-mediated immunity studies of persons exposed during a terrorist attack on the United Senate Office Building suggested possible subclinical infection in exposed persons who had received antibiotic prophylaxis but did not develop symptoms.[52]

Detailed data concerning immunity following inhalational anthrax are not available because of the rarity of this form of the disease and the high fatality rate. In studies of the several persons who have recovered during the past 25 years, low levels of antibodies were detected following recovery from the clinical disease.

8. Patterns of Host Responses

8.1. Clinical Features

8.1.1. Cutaneous Anthrax. Typically, cutaneous anthrax occurs on the exposed parts of the body. Although a single cutaneous lesion is typical, some patients develop multiple discrete lesions. The lesion begins innocuously with a small papule that the patient may first notice because of pruritus. The papule develops after several days into a small vesicle or, occasionally, into several vesicles that then coalesce to form a ring. This area may be surrounded by a small ring of erythema and possibly some edema. The pruritus may continue, but there is no pain unless

secondary infection or a significant degree of local edema is present. A small dark area can be seen beneath the center of the vesicle or in the central area if a ring of vesicles has formed. Eventually, the vesicle or vesicular ring ruptures, discharging a clear fluid and revealing a depressed black necrotic central area known as an eschar. After 1–2 weeks, the lesion dries and the eschar begins to loosen and shortly thereafter separates, revealing a permanent scar.

The lesion is usually 1–3 cm in diameter and remains round and regular. Occasionally, a lesion may be larger and irregularly shaped. There may be regional lymphangitis and lymphadenopathy and some systemic symptoms such as a slight low-grade fever, malaise, and headache. Antibiotic therapy will not change the natural progression of the lesion itself; however, it may decrease or inhibit development of edema and systemic symptoms. Clinical progression may lag behind bacteriological cure by 24–48 h.[53]

Occasionally, the cutaneous reaction is severe and is characterized by significant local and spreading edema associated with blebs, bullae, induration, chills, and fever. This type of reaction is referred to as malignant edema. Whether malignant edema results from increased pathogenicity or dosage of the organism or because of host factors is unknown.

There have been reports of multiple simultaneous lesions on a patient, but laboratory confirmation of each lesion has been lacking.[53]

Lesions in specific body sites occasionally result in more severe local reactions. Several cases have been seen of anthrax involving tissue surrounding the eye in which extensive edema spread over the entire face and extended to the neck and upper thorax. In one such patient, edema involved his entire face and extended down to the upper part of the thorax. In this instance, the spread of the toxin resulted in an extensive cutaneous lesion with necrosis of both eyelids. Plastic surgery was necessary to repair the tissue damage of the eyelids and surrounding tissue. Plastic surgery may also be necessary for other significant lesions.

Infrequently, meningitis may develop as a complication of cutaneous anthrax (see Section 8.1.4).

8.1.2. Inhalational Anthrax. This form shows a biphasic clinical pattern with a benign initial phase followed by an acute, severe second phase.[26,54] The initial phase begins as a nonspecific illness consisting of malaise, fatigue, myalgia, mild fever, nonproductive cough, and, occasionally, a sensation of precordial oppression. Findings of the physical examination are essentially within normal limits except that rhonchi may be present. The illness may resemble a mild upper-respiratory-tract infection such as a cold or the "flu." After 2–4 days, the patient may show signs of improvement. However, there is then the sudden onset of severe respiratory distress with dyspnea, cyanosis, respiratory stridor, and profuse diaphoresis. In several cases, subcutaneous edema of the chest and neck has been described. The pulse, respiratory rate, and temperature become elevated. Physical examination reveals moist, crepitant rales over the lungs and possibly evidence of pleural effusion. Shock may develop. X-ray examination of the chest may reveal widening of the mediastinum and/or pleural effusion. Septicemia and meningitis may develop. Without rapid treatment with antibiotics respiratory support, and attention to the electrolytes, death occurs within 24 h after the onset of the acute phase. Among the 16 survivors of the bioterrorist attacks, including six who recovered from inhalational anthrax, nine who recovered from a cutaneous lesion were studied approximately 1 year after their illness. They continued to report significant health problems and poor life adjustment that impacted on their quality of life.[55] A review of 82 inhalational anthrax cases from 1900 to 2005 summarizes the clinical data available from the patients' records.[23] There is a report of an inhalational anthrax-like illness due to infection with *Bacillus cereus* strains carrying the pX01 and pX02 plasmids.[56]

8.1.3. Gastrointestinal Anthrax. There are two clinical presentations for disease resulting from ingestion of *B. anthracis*: abdominal and oropharyngeal.[57,58] The symptoms of abdominal anthrax are initially nonspecific and include nausea, vomiting, anorexia, and fever. With progression of the disease, abdominal pain, hematemesis, and bloody diarrhea develop. Ascites may be present. Occasionally, the symptoms and signs resemble an acute surgical abdomen, which in some cases has resulted in surgery. With further progression, toxemia develops with shock, cyanosis, and death. The time from onset of symptoms to death has most frequently varied from 2 to 5 days. In oropharyngeal anthrax (also referred to as cervical anthrax), patients have experienced fever, submandibular edema, cervical lymphadenopathy, and anorexia. Some reports describe the presence of acute inflammatory lesions in the oral cavity and/or oropharynx. The mortality rate for gastrointestinal anthrax has been reported to vary from 25 to 60%.

8.1.4. Other Forms. Meningitis, seen in less than 5% of anthrax cases, may be a complication of any of the three forms of primary anthrax infection. In the bioterrorist attack, meningitis was seen in half of the inhalational cases. Rarely, meningitis is reported without a known primary site of infection. Symptoms of meningeal anthrax develop one to several days after the onset of the primary lesion and are similar to those associated with hemorrhagic meningitis. Death, if it occurs, usually does so in 1–6 days after onset of disease. Septicemia is only rarely seen in patients with treated cutaneous lesions; it is more commonly seen in patients with inhalational and gastrointestinal anthrax.

8.2. Diagnosis

A source of exposure to the infectious agent is of importance in considering a diagnosis of anthrax. Only rarely have cases occurred for which the source of infection could not be identified.

Cutaneous anthrax should be suspected when an individual describes a painless, pruritic papule, usually on an exposed part of the body. Vesicular fluid should reveal *B. anthracis* organisms microscopically and on culture. The differential diagnosis should include contagious pustular dermatitis (ecthyma contagiosum or orf), milker's nodule, plague, staphylococcal disease, and tularemia.

The initial symptoms of inhalational anthrax are nonspecific and may resemble those of an upper-respiratory-tract infection. Characteristically, with the sudden development of the acute phase, there is severe respiratory distress and radiographic examination of the chest should reveal widening of the mediastinum, a typical occurrence with inhalational anthrax (see http://anthrax.radpath.org). The acute phase resembles diseases that result in respiratory failure and shock.

In gastrointestinal anthrax, the patient presents with signs and symptoms of gastroenteritis. Organisms may be demonstrable in vomitus and feces from the infected individual. The differential diagnosis includes diseases that cause moderately severe gastroenteritis, such as shigellosis and *Yersinia* gastroenteritis. In the cervical form, the signs and symptoms might suggest pharyngitis, such as seen with streptococcal infections.

In anthrax meningitis, in most cases, there is a primary site of infection. CSF should contain *B. anthracis*. In septicemia, blood cultures should be positive for *B. anthracis*.

Several rapid diagnostic tests have been developed for specific diagnosis during the acute-phase illness. Toxin can be detected in clinical specimens using a capture ELISA or a chromatographic assay. Suitable specimens for toxin assay are vesicular fluid or lesion exudate in cutaneous cases, serum in gastrointestinal and inhalation cases, and CSF in meningitis cases.[14,59] Polymerase chain reaction detection of *B. anthracis*, whether living or dead, is possible utilizing primers targeting pX01 and pX02 plasmid DNA sequences.[11] Fluorescent-antibody staining of clinical and autopsy specimens is an older approach that has been updated through the use of monoclonal antibodies against cell wall and capsule components unique to *B. anthracis*.[8,14]

Serological tests currently being used in the United States include ELISA and electrophoretic immunotransblot (EITB).[51] Both tests are based on detecting components of the anthrax toxin. The ELISA test is used primarily for screening and is less specific than the EITB. The EITB can be modified to demonstrate titer changes between acute and convalescent serum samples.[9]

9. Control and Prevention

9.1. General Concepts

The risk of industrial infection has been reduced significantly over the past years as the use of imported animal products has been reduced because of changing business conditions, the increased use of synthetic materials, and the use of human vaccine (see Section 9.3). If imported materials for industrial use again become a problem, then disinfecting the animal products either before they are imported or when they enter the United States is important. Washing with formaldehyde, as was done at the Liverpool Disinfection Station in England for many years, could be used; other methods that could be instituted are ethylene oxide treatment, irradiation, or autoclaving. However, because imported animal products may enter the United States through a number of ports, and due to complexities of these decontamination procedures, development of a central facility in which to process the imported materials would be very difficult.

In the United States, improvements in industrial hygiene have been of some benefit in reducing the exposure of the worker to infectious materials and aerosols. The most important are the use of dust-collecting equipment during the initial processing cycle and institution of effective environmental cleanup procedures.

Employees should be educated about the disease and the recommendations for working in a contaminated environment and for reducing the risk of developing the disease. All employees should be vaccinated. Medical consultation services should be available to the employees. Adequate cleanup facilities and clothes-changing areas should be available so that workers do not wear their contaminated clothes home.

Gastrointestinal anthrax can be prevented by forbidding the sale of meat for consumption from sick animals or animals that have died from disease. Depending on the circumstances, it may be important to alert individuals who may come in contact with contaminated meat about the disease and about the need to cook all meats thoroughly.

All cases or suspected cases of anthrax should be reported to local public health officials so that appropriate epidemiological investigations can be conducted. Additional cases in humans or animals may be prevented by the prompt reporting and investigation of circumstances surrounding every case.

In the United States, agricultural anthrax is not a significant problem. This reflects the high standards of animal husbandry practiced in this country. Animals that graze in areas known as anthrax districts should be vaccinated annually with the Sterne strain animal vaccine. All animals suspected of dying from anthrax should be examined microbiologically; blood or tissue smears can be examined microscopically, and cultures can be set up from these same materials. All animals that have died with a confirmed diagnosis of anthrax should be thoroughly burned and the remaining bones and other materials buried deeply. Animals that die with a suspected diagnosis of anthrax can be cultured using a sample of blood. The carcass should then be handled as outlined above. If incineration is not possible, then the carcass should be covered with lime and buried at least 6-ft deep to discourage it being uncovered by scavenging animals.

9.2. Antibiotic Prophylaxis and Chemotherapeutics

Prophylactic antibiotics or chemotherapeutic agents are not used for anthrax except in three situations. If an individual has been inoculated with a live Pasteur-type spore animal anthrax vaccine, prophylactic penicillin should be given. Additionally, prophylactic antibiotics should be considered if a person is known to have eaten contaminated meat. The third situation is when an individual has been exposed to a high dose of *B. anthracis*. In these instances, post-exposure prophylaxis (PEP) is given to kill any *B. anthracis* organisms that may have been inoculated, ingested, or inhaled, and thus prevent the production of toxin. Persons with gastrointestinal or aerosol exposure should receive a 60-day course of antibiotics and be concurrently immunized with the human anthrax vaccine.[60] Laboratory studies with primates have evaluated the effectiveness of post-exposure prophylaxis and vaccination for inhalational anthrax. Protection was demonstrated using penicillin, ciprofloxacin, or doxycycline, and for doxycycline and vaccination.[61] When prophylactic antibiotics are given, the patient should be kept under surveillance for 10 days after initial therapy.

9.3. Immunization

There is an effective anthrax vaccine that was field tested in employees of four different textile mills in the United States, which demonstrated an effectiveness of preventing cutaneous anthrax of 92.5%.[62,63] The vaccine is a precipitate from a cell-free culture filtrate and contains protective antigen. This vaccine should be used for all employees who may be exposed to contaminated materials or environment. Additionally, anyone who comes into a mill-processing *B. anthracis*-contaminated material should also be vaccinated. Currently, the vaccine is given subcutaneously with three doses given at 2-week intervals followed by three booster inoculations at 6-month intervals and then annual booster inoculations. Alternate immunization schedules and routes of administration have been evaluated, and changes in the current recommendations may be adopted. Serological testing of vaccines has found a significant serological response to protective antigen beginning about 7 days after receipt of the second dose. Veterinarians, laboratory personnel, and other persons who, because of their occupation, have ongoing risk for contact with anthrax should also be immunized with the human anthrax vaccine. The human anthrax vaccine, BioThrax® is available on a limited basis. Those interested should contact Emergent Biosolutions, 2273 Research Boulevard, Suite 400, Rockville, Maryland 20850. Several new human vaccines are under development.

9.4. Immune Serum

In the 1800s and into the 1900s, hyperimmune serum was produced in horses and used to treat patients with cutaneous anthrax with some successes as reported in the literature. The hyperimmune serum was produced in Italy. Currently, anthrax globulin from anthrax vaccinees is available for inhalation anthrax therapy trials under an Investigational New Drug application with the Food and Drug Administration.

10. Unresolved Problems

The ecology of *B. anthracis* is poorly understood. Some investigators hold that *B. anthracis* is an obligate mammalian pathogen which persists in contaminated soil due to a highly resistant spore, but others postulate that *B. anthracis* has a growth phase in soil which compounds the risk posed by spore persistence. The latter hypothesis holds that the anthrax bacillus may utilize soil as a growth medium or perhaps replicate within components of the soil fauna such as nematodes or free-living amebae. The timing of animal anthrax outbreaks is difficult to explain solely on the basis of soil persistence, but efforts to grow the agent in soil have not produced convincing results. Certain soil factors such as pH and calcium content appear to be associated with soil districts that remain infectious for livestock for many years, but whether these factors support growth or simple spore persistence is unresolved. A better understanding of anthrax ecology is a key to more effective intervention.

Wildlife anthrax continues to be a problem, particularly in developing countries. Improved control measures are needed to preserve the affected species, minimize associated public health risks, and reduce potential environmental

contamination. Due to poor understanding of the risk factors contributing to wildlife anthrax, epidemiologic investigations are urgently needed to develop science-based control programs.

The major anthrax virulence factors have been well characterized in recent years. Less well studied are potential lesser virulence factors such as enterotoxin, and their role in anthrax pathogenesis.

The protective antigen-based vaccine that is currently approved for administration to humans in the United States provides good protection but requires multiple doses and does not result in rapid development of immunity. A recombinant protective antigen-based vaccine is being developed and evaluated, but this vaccine has similar problems. Vaccine development remains a priority. Also, the role of antiserum for post-exposure prophylaxis and case treatment needs more extensive evaluation. Early animal studies suggest that antiserum has a potential role if administered early in the course of the disease.

Post-exposure prophylaxis (PEP) clearly has a role in anthrax prevention, particularly in terrorist attacks. However, optimal practices remain to be defined. Which antimicrobial agents should be used? What is the optimal duration for PEP? What regimen should be followed in situations where post-exposure vaccination is also feasible? How should a general PEP recommendation be modified for special populations such as in persons with immunodeficiency and the aged? Can the same PEP recommendations be used for persons with cutaneous, intestinal, and inhalational exposure?

The extent, if any, of potential long-term sequelae of anthrax infection needs better definition. In cutaneous anthrax, surgical correction of problems arising due to scarring is occasionally necessary. However, limited information is available on other potential long-term effects of inhalational or gastrointestinal anthrax. A 1-year follow-up of anthrax survivors of the 2001 bioterrorist attacks revealed continuing psychological distress and reduced health-related quality-of-life indices, pointing to the need for further study.

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CHAPTER 6

Bacterial Foodborne Disease

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1. Introduction

Foodborne disease comprises a broad group of illnesses caused by the consumption of foods contaminated with toxic substances or pathogenic microorganisms. The agents responsible for foodborne illnesses can be divided into four general categories: chemical (e.g., mushroom or scombroid poisoning), parasitic (e.g., *Trichinella* or *Giardia*), viral (e.g., Hepatitis A or Norovirus), and bacterial (e.g., *Salmonella* or *Campylobacter*). Only the bacterial will be considered in this chapter.

Because there are many foodborne bacterial pathogens, it is useful to categorize them further. Epidemiologically, one of the most useful methods is to divide these according to their reservoir (Table 1). *Campylobacter*, Shiga toxin-producing *Escherichia coli* (STEC) (the most common of which is *E. coli* O157:H7; also known as enterohemorrhagic *E. coli*, or EHEC), nontyphoidal *Salmonella*, and *Yersinia* often live as commensals in the intestinal tracts of animals. They are transmitted to humans largely through beef (STEC and *Salmonella*), pork (*Yersinia*), poultry (*Salmonella* and *Campylobacter*), and eggs (*Salmonella* serotype Enteritidis). *Brucella* differs from the others in this group in that it usually acts as a pathogen in animals; it is transmitted from the animal reservoir to humans via the consumption of milk as well as via other routes. *Vibrio, Aeromonas*, and *Plesiomonas* species all inhabit aquatic environments and are transferred to humans through seafood (*V. parahaemolyticus, Aeromonas*, and *Plesiomonas*) or through water (*V. cholerae*, *Aeromonas*, and *Plesiomonas*).[1] Enterotoxigenic *E. coli* (ETEC), *Shigella*, *Salmonella typhi*, and *Staphylococcus* are species that are transmitted primarily from infected handlers through food. Finally, there is a group of organisms, including *Listeria, Clostridium perfringens, Clostridium botulinum*, and *Bacillus cereus*, that generally originate in environmental sources.

Alternatively, organisms can be classified according to the three main ways by which they produce illness: toxin production prior to consumption, toxin production in vivo, and invasion of the host (Table 2). Differentiation into the last two categories has become complicated in light of discoveries of genes for type III and type IV secretion systems in a variety of bacteria. These secretion systems inject bacterial virulence factors directly into host cells, allowing for activation of several biological functions that can eventually result in cell invasion. Despite this evidence, general assumptions about the predominant mechanism of bacterial pathogenesis can be made based on incubation period. Organisms in the first category, *C. botulinum, B. cereus* (emetic-type illness), and *Staphylococcus*, produce toxins as they grow in food. Because these toxins are completely formed by the time the food is consumed, vomiting or diarrhea due to the latter two have a short incubation period (1–6 h). As one might predict, the toxins produced by these organisms are generally resistant to stomach acid and to proteases found in the gastrointestinal tract. Organisms in the second category, *B. cereus* (diarrheal type illness), *C. perfringens*, and "toxigenic" *V. cholerae*, have an incubation period of 6 h to several days after ingestion and during this time are thought to elaborate toxins as they multiply within the gastrointestinal tract. The last category includes organisms that have either demonstrated adherence and invasion of cultured cells or have been discovered with type III or type IV secretion systems: *Aeromonas, STEC*, enteropathogenic *E. coli* (*EPEC*), *Brucella, Campylobacter, Listeria*, nontyphoidal *Salmonella,*

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Table 1. Foodborne Pathogens, Classified by Reservoir

Animal reservoir (vehicles include meat, poultry, and eggs)	*Brucella*
	Nontyphoidal *Salmonella*
	Campylobacter
	Yersinia
	EHEC
Marine organisms (vehicles include seafood and water)	*Vibrio* species
	Aeromonas
	Plesiomonas
Human reservoir (transmitted through food contaminated by infected or colonized food handlers)	ETEC
	Staphylococcus
	Salmonella typhi
	Shigella
Other environmental (can be found in soil, water, or a variety of foods)	*C. botulinum*
	C. perfringens
	B. cereus
	Listeria

Salmonella typhi, Shigella, V. vulnificus, *V. parahaemolyticus*, and *Yersinia*. The incubation period for these organisms can be even longer, lasting from a few hours to several weeks. As stated earlier, these categories are not mutually exclusive. The pathogenesis of many foodborne pathogens is still incompletely understood, and many of the invasive organisms also elaborate toxins that may be important in their ability to cause disease.

Most of the bacteria that cause foodborne illness are discussed in separate chapters in this book (see Chapters 6, 7, 8, 11, 14, 21, 31, 32, 42, and 43). Because illness due to six organisms, *S. aureus, B. cereus, C. perfringens*, noncholera vibrios, *Aeromonas*, and *Plesiomonas*, is not discussed explicitly elsewhere, this chapter is devoted to these pathogens.

Table 2. Classification of Bacterial Foodborne Pathogens According to Pathogenesis

Organisms that cause disease by production of toxins in food prior to consumption	*B. cereus* (emetic type)
	C. botulinum
	Staphylococcus
Organisms that produce disease by production of toxins after ingestion	*B. cereus* (diarrheal type)
	C. perfringens
	Plesiomonas
	V. cholerae
Organisms that produce disease by invasion of the host	*Aeromonas*
	Brucella
	Campylobacter
	EHEC
	EPEC
	Listeria
	Nontyphoidal *Salmonella*
	Salmonella typhi
	Shigella
	V. paraheaemolyticus
	V. vulnificus
	Yersinia

2. Historical Background

In the United States formal surveillance for diarrheal illness began after World War I when state and territorial health officers, concerned about high morbidity and mortality rates caused by typhoid fever and infantile diarrhea, recommended that cases of enteric fever be investigated and reported. In 1923, the United States Public Health Service published summaries of outbreaks of gastrointestinal illness attributed to milk. Outbreaks caused by all foods were added in 1938. These activities were instrumental in the enactment of public health measures that have decreased the incidence of enteric disease.

In 1961, the Communicable Disease Center [which later became the Centers for Disease Control and Prevention (CDC)] assumed responsibility for collecting and publishing reports on foodborne illness. The current system of surveillance of foodborne disease outbreaks began in 1966, by incorporating into an annual summary all reports of such outbreaks in the United States.

The National Notifiable Diseases Surveillance System has been collecting data on sporadic illness (i.e., illness not associated with an outbreak) since 1912. In that year the Public Health Service began recommending immediate telegraphic reporting of five infectious diseases and monthly reporting of ten others. Since then, the systems have expanded to include 59 infectious diseases and is currently administered by the CDC (see Section 3.2).

In 1996, the Foodborne Diseases Active Surveillance System (FoodNet) was established as the foodborne disease component of the CDC's Emerging Infections Program (EIP). FoodNet is collaborative effort between the CDC, the U.S. Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), and ten EIP sites. Initially, FoodNet collected data on the incidence of laboratory-confirmed cases of *Campylobacter*, STEC including *E. coli* O157, *Listeria, Salmonella, Vibrio,* and *Yersinia* in five EIP sites (Minnesota, Oregon, and portions of California, Connecticut, and Georgia). The catchment area has since been expanded from those five sites (13.2 million people) to ten sites constituting over 44 million people (approximately 15% of the U.S. population). In addition to bacteria, FoodNet also performs active surveillance for two parasitic organisms, *Cryptosporidium* and *Cyclospora*, and a disease condition called hemolytic uremic syndrome (HUS), which can be an indicator of enterohemorrhagic *E. coli* or other shiga toxin-producing pathogen infection.

3. Foodborne Disease Outbreak Surveillance

Much of our knowledge about the epidemiology of foodborne illness comes from the investigations of outbreaks. Such investigations are initiated by state and local health departments in response to requests by consumers or physicians or because of clustering of reported illnesses. Although clustering is typically defined as increased spatial density of cases, it may also be defined as increased incidence of cases from which bacterial isolates are genetically identical. Prior to 2001, outbreaks were reported to the CDC through the Foodborne Disease Outbreak Surveillance System. However, in 2001, the CDC implemented the Electronic Foodborne Outbreak Reporting System (eFORS). Reports filed through eFORS contain data on demographics of the cases, a description of the outbreak, proposed etiology and food source. Use of eFORS has resulted in a substantial increase in the number of reported outbreaks. Note: In 2009, eFORS was renamed the National Outbreak Reporting System (NORS) to reflect the added ability to report waterborne, zoonotic, and person to person transmission of enteric diseases.

The CDC has published guidelines for the confirmation of foodborne outbreaks caused by several etiologic agents.[2] Table 3 contains some examples of guidelines for a few pathogens. In order to confirm the etiology of an outbreak, specific criteria must be met. An outbreak of *B. cereus* emetic-type illness, for example, is considered confirmed if the organism is isolated from at least two cases but not from controls or if the organism can be found in numbers greater than 10^5/g in the implicated food.

Summaries of the numbers of outbreaks and outbreak-related cases during a 12-year period are shown in Table 4. The figures come from a report from the Center for Science in the Public Interest (CSPI) that compiles CDC outbreak reports along with state outbreak reports and information from scientific and medical journals and newspaper articles. These numbers represent only a small, unrepresentative portion of the actual foodborne illness in the United States, because most outbreaks go unrecognized and because most foodborne illnesses are sporadic. Several factors determine the likelihood that any given outbreak will be recognized and investigated:

1. Size of the outbreak. Larger outbreaks are more likely to be recognized and investigated. Furthermore, larger outbreaks are most likely to result in useful data on etiology and food source. A study of outbreaks occurring within the FoodNet catchment area between 1998 and 1999 observed that 37% of outbreaks with ten or more cases had an etiologic agent identified but only 23% of outbreaks with fewer than ten cases had an etiologic agent identified.[3]
2. Severity of illness. Outbreaks of severe illness are more likely to be recognized and investigated. An outbreak of *C. perfringens* diarrhea, which causes relatively mild illness, will likely go unnoticed unless a large number of illnesses occur. Any case or outbreak of botulism, on the other hand, is likely to come to the attention of public health authorities. During the years from 1990 to 2002, the average size of *C. perfringens* outbreaks was 62 cases, whereas that of botulism outbreaks was just 4 cases (Table 4).
3. Incubation period. A short incubation period increases the likelihood that an outbreak will be recognized and a vehicle implicated. Illness due to *S. aureus*, for example, may be relatively easy to recognize as a common-source problem because of its short incubation period (usually 2–4 h). An outbreak of listeriosis, however, may go undetected because its incubation period is several weeks.
4. Setting. Outbreaks that occur in groups of persons that are clustered geographically are more likely to be recognized. Outbreaks occurring in schools or camps, for example, are more likely to be recognized than outbreaks occurring in the community. However, the active surveillance within the FoodNet catchment area and the availability of molecular typing methods have increased the ability to detect outbreaks that are not geographically clustered. Several studies have utilized pulsed-field gel electrophoresis to identify multi-state outbreaks, which otherwise might not have been detected.[4–6]
5. Ease of diagnosis. The identification of certain organisms, such as vibrios or STEC, requires special techniques that may not be routinely performed when physicians request stool cultures. For this reason, outbreaks of these organisms may not be recognized.
6. Resources available to the local and state health departments. Even large outbreaks can go unrecognized if health departments are unable to devote resources to investigating them. A 1994 outbreak of *Salmonella* serotype Enteritidis involving tens of thousands of cases in over 30 states served to highlight this. Although this outbreak was huge by anyone's standards, the cases were widely dispersed geographically, and it was not immediately apparent that an outbreak was occurring. It was identified only because the Minnesota State Health Department had an aggressive program of serotyping *Salmonella* isolates sent to them, which allowed them to detect the sudden increase in one specific serotype.

There are several limitations of the current system for reporting outbreaks of foodborne disease.[3,7] First, not all outbreaks are included in the surveillance report. Outbreaks occurring on cruise ships, resulting from indirect transmission via contaminated surfaces or from foods eaten outside of the United States are excluded. Furthermore, the classification of food vehicles does not account for multi-ingredient foods (e.g., contained milk can be consumed independently or as an ingredient in a myriad of dairy products). Since the true numbers of outbreaks and cases are probably underestimated to a high degree by such a system, it cannot be used to estimate incidence. Also, because the degree of underreporting is not consistent from pathogen to pathogen, data from this system cannot be used to draw conclusions about the *relative* incidence of these illnesses. Trends are not always reliably noted by this system either; a decrease in the total number of foodborne out-

Table 3. Criteria for Confirmation of Foodborne Outbreaks Due to *B. cereus, C. perfringens, S. aureus, V. cholerae*, and *V. parahaemolyticus*[a]

Etiologic agent	Incubation period	Clinical syndrome	Confirmation
B. cereus			
Emetic-type illness	1–6 h	Vomiting, sometimes diarrhea	Isolation of organism from stools of ≥2 ill persons and not from stools of controls OR Isolation of $\geq 10^5$ organisms/g from epidemiologically implicated food, provided specimen properly handled
Diarrheal illness	6–24 h	Diarrhea, abdominal cramps, sometimes vomiting (fever is rare)	(Same as above)
C. perfringens	6–24 h	Diarrhea, abdominal cramps (vomiting and fever are uncommon)	Isolation of $\geq 10^6$ organisms/g in stool of ≥2 ill persons, provided specimen properly handled OR Demonstration of enterotoxin in stools of ≥2 ill persons OR Isolation of $\geq 10^5$ organisms/g from epidemiologically implicated food, provided specimen properly handled
S. aureus	30 min to 8 h; usually 2–4 h	Vomiting and diarrhea	Isolation of organisms of same phage type forming stool or vomitus from ≥2 ill persons OR Demonstration of enterotoxin in epidemiologically implicated food OR Isolation of $\geq 10^5$ organisms/g from epidemiologically implicated food, provided specimen properly handled
V. cholerae, nonepidemic	1–5 days	Watery diarrhea	Isolation of organisms of same serotype from stools of ≥2 ill persons
V. parahaemolyticus	4–30 h	Diarrhea	Isolation of Kanagawa-positive organisms from stools of ≥2 ill persons OR Isolation of $\geq 10^5$ Kanagawa-positive organisms/g from epidemiologically implicated food, provided specimen properly handled

[a]From CDC Surveillance Summaries.[2]

breaks in the mid-1980s, for example, may have been due to the diversion of public health resources to combat the burgeoning acquired immunodeficiency syndrome (AIDS) epidemic.[8]

Given these limitations, it is not surprising that a significant portion (28.3%) of CSPI-collated outbreaks are of unknown etiology (Table 4). Sixty-eight percent of outbreaks reported to the CDC between 1998 and 2002 had an unknown etiology.[7] In many cases, etiologies may have been suspected by the investigators but not confirmed according to the CDC's criteria. A study of outbreaks occurring in FoodNet sites between 1998 and 1999 reported that 71% of outbreaks did not have an etiologic agent identified.[3] Thus, even with increased surveillance capabilities, the challenges of gathering data associated with foodborne outbreaks are daunting. It is possible that there are other etiologic agents that have yet to be discovered. It is also possible that a large portion of these outbreaks are due to viruses, which remain particularly difficult to diagnose.[7]

Nonetheless, information from this system has been invaluable in the identification and removal of contaminated products from the market, the determination of factors contributing to foodborne illness, the development of public health strategies to prevent foodborne illness, the evaluation of effectiveness of regulatory response, and the advancement of our understanding of the pathogenesis and clinical characteristics of foodborne illness.

Table 4. Summary of Confirmed and Suspected Foodborne Outbreaks and Outbreak-Related Cases in the United States from 1990 to 2002[a]

	Total outbreaks	Percent of total	Total cases	Percent of all cases	Cases per outbreak
B. cereus	60	1.5	1,441	1.1	24
C. botulinum	56	1.4	207	0.2	4
C. perfringens	224	5.5	13,951	10.9	62
Campylobacter	75	1.9	2,023	1.6	27
E. coli	228	5.6	7,227	5.7	32
Listeria	11	0.3	319	0.3	29
S. aureus	175	4.3	5,305	4.2	30
Salmonella	975	24.1	42,288	33.1	43
Shigella	68	1.7	4,610	3.6	68
Streptococcus	3	0.1	226	0.2	75
V. cholerae non-O1	4	0.1	20	0.02	5
V. cholerae O1	4	0.1	34	0.03	9
V. parahaemolyticus	40	1.0	920	0.8	23
V. vulnificus	1	0.02	2	0.0	2
Yersinia	10	0.3	112	0.1	11
Other bacteria	10	0.3	232	0.2	23
Two or more etiologies	27	0.7	756	0.6	28
Total bacteria	1,971	48.6	79,673	62.4	40
Total viral	423	10.4	20,072	15.7	47
Total parasitic	52	1.3	3,996	3.1	77
Total chemical	460	11.4	2,694	2.1	6
Unknown etiology	1,146	28.28	21,300	16.68	19
Total	4,052	100.00	127,735	100.00	32

[a]From CSPI Outbreak Alert.[345]

3.1. Surveillance of Sporadic Diarrheal Illness

Prior to 1995, data on sporadic diarrheal illness was largely obtained through passive surveillance systems. The National Notifiable Diseases Surveillance System (NNDSS) collects data on several illnesses that are sometimes foodborne: botulism, brucellosis, cholera, *E. coli* O157:H7, salmonellosis, shigellosis, and typhoid fever. The data provided are numbers of cases, location, and patient demographic characteristics. Because the NNDSS records both sporadic and outbreak-related illnesses, the numbers of cases registered by this system are typically much higher than those recorded by the outbreak surveillance system; in 2003, for example, the outbreak surveillance system reported 3,948 cases of salmonellosis (351), whereas NNDSS reported 43,657 cases of this disease.[9] There is uncertainty in the number of the NNDSS salmonellosis cases that are due to foodborne transmission, although reports suggest this number is 80–95%.[10]

Presently, FoodNet is the primary active surveillance system used to identify sporadic cases of foodborne illness. FoodNet grew out of CDC's Emerging Infections Program (EIP) and has since expanded to ten sites. FoodNet monitors incidence of foodborne disease and uses active laboratory surveillance to collect data on etiology, risk factors, and outcomes of foodborne disease caused by *Campylobacter*, STEC, *Listeria, Salmonella, Vibrio*, and *Yersinia*, and two parasitic organisms, *Cryptosporidium* and *Cyclospora*. FoodNet personnel contact laboratories within catchment areas either weekly or monthly. The 2003 FoodNet rates are presented in Table 5.

Despite the much improved surveillance and data quality, there are still several limitations of the FoodNet methodology, some of which are due to reliance on laboratory-confirmed cases. Similar to passive surveillance, it is believed that case numbers are subject to a very high degree of underreporting. For example, estimates of salmonellosis indicate that the true incidence in the United States is approximately 40 times that reported to the CDC.[10,11] Also, isolation of pathogens does not prove that they cause disease. As discussed later, some bacteria are commensal in the human host. Furthermore, active FoodNet surveillance does not provide data on viral pathogens, since clinical laboratories (on which the system is based) do not routinely screen for foodborne viruses.

In addition to NNDSS and FoodNet, specific surveillance systems are operational and collect data on *Listeria, Vibrio* species, and *E. coli* O157:H7. These systems have

Table 5. 2003 FoodNet Incidence by Pathogen and Site[a]

	CA	CO	CT	GA	MD	MN	NY	OR	TN	All states
Campylobacter	27.1	14.69	15.59	7.16	7.68	18.52	11.88	16.24	7.81	12.6
Cryptosporidium	0.55	0.44	0.57	1.38	0.34	3.06	1.21	0.98	0.72	1.09
Cyclospora	0	0	0.11	0.09	0.04	0	0.03	0	0	0.03
E. coli O157	0.9	1.46	1.06	0.26	0.29	2.63	1.23	2.42	0.58	1.06
STEC, Non-O157	0	0.08	0.72	0.02	0	0.24	0.03	0.08	0.03	0.11
*STEC, O-Ag Undet**	0	0	0.03	0.05	0.07	0.2	0.08	0	0	0.05
Listeria	0.53	0.24	0.63	0.38	0.51	0.12	0.28	0.14	0.19	0.33
Salmonella	14.75	9.86	11.51	23.18	14.49	11.44	9.94	10.62	12.89	14.43
Shigella	8.62	9.3	2.01	13.2	8.48	2.04	5.99	2.92	6.86	7.27
Vibrio	0.62	0.04	0.32	0.32	0.42	0.08	0.18	0.17	0.17	0.26
Yersinia	0.59	0.2	0.46	0.56	0.22	0.24	0.38	0.14	0.5	0.39

[a] Adopted from 2003 CDC FoodNet Report.[12]

included active surveillance components as well as case–control studies designed to determine the routes of transmission of such diseases.

4. Etiologic Patterns

Despite previously described methodological limitations, FoodNet has collected the most useful current data on the etiologic patterns of foodborne disease in the United States. The most recent FoodNet data is from 2008.[354]

There were 18,499 laboratory-confirmed illnesses caused by either *Campylobacter*, STEC 0157:H7, non-0157 STEC, *Listeria*, *Salmonella*, *Vibrio Yersinia*, *Cryptosporidium or Cyclospora* among FoodNet sites in 2008.[354] *Salmonella* infections had the highest incidence of 16.2 infections/100,000 population followed by *Campylobacter* and *Shigella* with 12.68 and 3.03 infections per 100,000 population, respectively.[354] Because FoodNet active laboratory surveillance monitors only nine foodborne pathogens, the rank in foodborne morbidity differs from passive surveillance data (Table 4). According to passive surveillance data, *C. perfringens*, *E. coli*, and *S. aureus* are ranked second, third, and fourth in number of cases with bacterial etiology. Observation of incidence data since FoodNet was implemented in 1996 suggests that, in general, incidence there was a decreasing trend in incidence of foodborne disease through 2004, with rates "leveling off" since that time.

Incidence of foodborne illness appears to vary by a number of factors including seasonality, geographic region, and demographic variables. A large proportion of foodborne illnesses are reported during the summer months of June–August.[12] For example, a 2003 CDC FoodNet report stated that approximately half of all *Vibrio* and STEC infections and more than a third of *Salmonella* infections occurred within these 3 months.[12] In contrast, approximately 41% of *Yersinia* infections occurred during winter months.[12] Incidence of foodborne illness also varied by both age and gender with children less than 1 year of age and males typically having higher incidence of infection.[12]

FoodNet has also detected state-specific differences in isolation rates of foodborne pathogens (Table 5). For example, the 2003 CDC FoodNet Final Report shows that California has a high incidence of *Campylobacter*, which has remained consistently high (data not shown) in comparison to other FoodNet sites over the past decade. In contrast, Maryland and Georgia have consistently low *Campylobacter* infection rates. Many hypotheses have been generated as to the cause of this phenomenon, including different food, water, and environmental exposures, and different food processing regulations.[13,14]

Among laboratory-confirmed foodborne disease cases in 2003, 22% were hospitalized and 83 cases died.[12] Thus the case-fatality rate was very low overall other than *Listeria*, which had a 17% case-fatality rate.[12] Case-fatality ratios are generally low for all foodborne pathogens. The majority of deaths that occur are in the elderly or very young. However, *Listeria* is also a problem in the immunocompromised patient as well as in infants and otherwise healthy pregnant women. Hospitalization varied significantly by the infecting organism. Again *Listeria* cases were mostly likely to have been hospitalized (91%).

It is important to note that etiologic patterns may vary throughout the world. These patterns are dependent on many factors, such as food preferences, food preparation, physician and public awareness, and laboratory capabilities. In the United States salmonellosis currently comprises the largest percentage of foodborne illness cases.[15] In England and

Wales *Campylobacter* is the major etiologic agent of foodborne disease.[16] Japan, on the other hand, has very different etiologic patterns, probably related to many of the aforementioned factors. *Vibrio*-related gastroenteritis, first described in that country, is usually the dominant pathogen in foodborne outbreaks.[17] These associations generally reflect the different staples in diets around the world (i.e., higher seafood consumption in Japan, higher meat and poultry consumption in the United States and Europe). Clearly, some of these differences represent ascertainment bias; however, there is little doubt that different patterns exist in different regions of the world.

With this background, let us turn to a consideration of the specific agents *S. aureus, B. cereus, C. perfringens, Vibrio* species, *Aeromonas*, and *Plesiomonas*.

5. *Staphylococcus aureus*

5.1. Historical Background

Although Dack modestly credited others for the discovery of staphylococcal food poisoning, he and his coworkers were the first to prove that this illness was due to toxin production by *S. aureus*.[18] They performed classic experiments in volunteers, demonstrating that culture filtrates of staphylococci isolated from a cream-filled sponge cake that had been the implicated vehicle in a foodborne outbreak could cause gastroenteritis.[19]

It is generally recognized that *S. aureus* exotoxins are responsible for symptoms of skin infections, while ingested enterotoxins cause the diarrhea and vomiting associated with staphylococcal food poisoning. The work on the enterotoxins produced by *S. aureus* has been fundamental to our understanding of this syndrome. Dolman and Wilson.[20] established that antibody to the enterotoxin could be made and, 15 years later, Surgalla et al.[21] showed that there was more than one immunologic type. Casman, Bergdoll, and others characterized the physicochemical properties of these enterotoxins and initially established that there were seven serotypes: A, B, C_1, C_2, C_3, D, and E.[22] To date, as many as 13 other Staphylococcal enterotoxins and staphylococcal enterotoxin-like toxins have been identified through genome analysis.[23,24]

5.2. Microbiology

5.2.1. Biological Characteristics of the Organism. Staphylococci are gram-positive, nonencapsulated, nonmotile, non-spore-forming cocci that usually appear in grapelike clusters, hence the name. They are aerobic and facultatively anaerobic, may be β-hemolytic and catalase-positive, and ferment glucose. The genus *Staphylococcus* contains almost two dozen species that are traditionally divided into two groups on the basis of coagulase production. Coagulase-positive staphylococci are almost always *S. aureus*, although some strains of *S. intermedius* and *S. hyicus* also produce this enzyme.[22] All other species are coagulase-negative, the most important of which are *S. epidermidis* and *S. saprophyticus*.

S. aureus, the usual pathogen in foodborne disease, is capable of producing at least 21 enterotoxins, which share structure and sequence similarities. These known enterotoxins are all short proteins with molecular weights of 24–30 kD, which contain large numbers of lysine, aspartate, glutamate, and tyrosine residues.[24] They are heat stable and resistant to a variety of proteases, including pepsin and trypsin.

Superantigenic activity has been demonstrated for more newly characterized enterotoxins, but rarely emetic activity.[24] The mechanism of emesis-inducing action of staphylococcal enterotoxins is still unknown although their positive charge,[25] cysteine loop structure (found only in some enterotoxins, most notably SEA),[26] and disulfide bond formation[27] have been suggested as a link(s) to emetic activity. Since vagotomy and abdominal sympathectomy in monkeys render them completely immune to the emetic effects of these toxins, their receptor or receptors are probably within the lumen of the gastrointestinal tract. It is not clear what these receptors are or the precise binding sites in enterotoxin proteins with which they interact.[24] The mechanism by which these toxins produce diarrhea is even less well understood; they fail to cause a reaction in rabbit ileal loop assays, though one group has been able to demonstrate fluid secretion in rat small intestine when challenged with staphylococcal enterotoxin B.[28]

A variety of other toxic substances are produced by *S. aureus* that are hemolytic, leukocytic, dermonecrotic, and lethal to animals. All *S. aureus*, by definition, produce a coagulase that causes coagulation of plasma; it is both free and bound to the cell wall. *S. aureus* also produces a fibrinolysin, hyaluronidase, deoxyribonuclease, and various lipases. Many of these substances are physiologically important for the other manifestations of staphylococcal infections, but are beyond the scope of this chapter; the reader is referred to Chapter 34 of this book for further information.

S. aureus is not the only species in this genus that is capable of producing staphylococcal enterotoxins. Strains

of *S. intermedius, S. hyicus*, and *S. epidermidis* have been shown to produce these.[22] The latter has been implicated in a foodborne outbreak.[29] It is important to note also that not all *S. aureus* strains are enterotoxigenic. The frequency of enterotoxigenic strains in foods varies from one study to another.

5.2.2. Laboratory Diagnosis. The medium currently recommended by the FDA for isolation and enumeration of large quantities of staphylococci from food is an egg yolk–tellurite–glycine–pyruvate agar ("Baird-Parker agar").[30] Coagulase-positive staphylococci produce a zone of clearing in egg-yolk emulsion and reduce tellurite salt to tellurium, causing the staphylococci to form black colonies. Mannitol as the sole or major source of carbon in the medium, along with a high NaCl content, can also be used in a selective medium, although such conditions may inhibit the growth of heat-stressed staphylococci.[31] It has been shown that this growth inhibition can be overcome with catalase.[25] Small numbers of *S. aureus* (expected in outbreaks where little source is available or competing species are high) are isolated using the most probable number method, in which tryptic soy broth is inoculated with sample to increase enumeration of *S. aureus* prior to streaking on Baird-Parker agar. Coagulase tests can be done in a tube using brain heart infusion broth and rabbit plasma containing EDTA. A clotting reaction of 4+ is recommended for confirmation of *S. aureus.*[30] An outbreak in Japan due to uncharacteristic egg-yolk reaction-negative *S. aureus*[32] suggests that more than one biochemical procedure should be used to confirm *S. aureus* species. However, most recommended tests are insufficient in delineating enterotoxigenic and nonenterotoxigenic staphylococci.[30]

Demonstration of staphylococcal enterotoxin is an important tool in outbreak investigations, since reheated food may contain toxin but no viable staphylococci. Several methods have been described, including enzyme-linked immunosorbent assay (ELISA),[33,34] enzyme immunoassay (EIA),[35] latex agglutination,[34] and immunoblot.[36] Several commercial kits have been formulated for toxin detection.[37] The method used should be capable of detecting concentrations of less than 1 ng/g of food, since a dose of 1 μg toxin/100 g food is sufficient to produce symptoms.[38]

Oligonucleotide probes, multiplex polymerase chain reaction (PCR), microarray assays, pulsed-field gel electrophoresis (PFGE), and plasmid restriction analysis are also used to demonstrate the presence of toxin genes in staphylococcal isolates.[23,39–44] These DNA-based techniques are used infrequently, however, in areas where resources are limited.

5.3. Descriptive Epidemiology

5.3.1. Prevalence and Incidence. Table 4 summarizes *S. aureus* outbreak data from the United States from 1990 to 2002. There are approximately 30 cases per outbreak and approximately 15 outbreaks per year, with *S. aureus* contributing 4.2% of all foodborne cases in the United States. A summary of annual foodborne pathogen-specific estimates in England and Wales from 1996 to 2000 showed that *S. aureus* contributes to only 0.5% of total foodborne illness.[16]

5.3.2. Epidemic Behavior and Contagiousness. Staphylococcal foodborne outbreaks are characterized by explosive onset between 30 min and 8 h after consumption of a contaminated vehicle (usually 2–4 h, depending on the amount of toxin ingested).[19,45] Attack rates are usually quite high, since very small quantities of enterotoxin can cause illness.[19] Secondary cases are uncommon.

5.3.3. Geographic Distribution. There can be geographic clustering of cases. Because outbreaks are usually related to food contamination from human staphylococcal carriers, one generally finds localized cases disseminating from one person who has prepared meals for people in confined spaces (such as prisons or nursing homes). If contamination occurs closer to the farm end of the farm-to-fork spectrum (i.e., in products such as milk), contamination and hence outbreaks may be more widespread.

5.3.4. Temporal Distribution. Outbreaks from staphylococci can occur at any time of the year, but most outbreaks are reported during the warm weather months.[45,46] This is probably because higher ambient temperatures provide better conditions for bacteria to multiply when foods are contaminated.

5.3.5. Age, Gender, Race, and Occupation. There are no age, gender, racial, or occupational characteristics that are peculiar to staphylococcal food poisoning.

5.3.6. Occurrence in Different Settings. Staphylococci are carried by such a high percentage of the population that almost any food-preparation setting can be involved. Approximately 60% of the population are intermittent carriers of *S. aureus* and 20% are thought to be persistent carriers.[47] Most reported outbreaks are from large gatherings, i.e., prisons, schools, group picnics, clubs, and restaurants. There have been reports of hospital-acquired *S. aureus* infections that have caused acute outbreaks of foodborne gastroenteritis.[48]

5.3.7. Foods Implicated in Outbreaks. For several years after the recognition of staphylococcal food poisoning, cream-filled baked goods were the leading source of this illness in the United States. In recent decades, other proteinaceous foods, especially meat and meat products, have predominated. In the United States and Great Britain, ham

has been the most commonly implicated vehicle, which may be in part due to its relatively high salt content; *S. aureus* is very tolerant of salt, which increases its resistance to heat. In Japan *S. aureus* food poisoning is caused mostly by processed foods composed of rice, rather than raw meat and meat products.[49] Milk and milk products are responsible for most *S. aureus* cases in France.[50] Both raw and pasteurized milk have been implicated as vehicles in *S. aureus* foodborne outbreaks in other countries. Other vehicles have included potato salad, egg salad, cheese, canned foods, and seafood. Improper holding temperature is the most common behavioral factor in *S. aureus* outbreaks.[8]

5.4. Mechanisms and Routes of Transmission

The single most important source of staphylococcal contamination in foods is humans, where this organism can commonly be found on mucous membranes, skin, or nasal carriage.[47,48]

As the number of methicillin-resistant *S. aureus* (MRSA) nosocomial and community-acquired infections grows, there may be a subsequent increase in MRSA gastroenteritis cases. MRSA strains are also capable of producing enterotoxins, however, these strains are likely to go unnoticed because *S. aureus* isolates from foodborne outbreaks may not be tested for antibiotic susceptibility.[48] MRSA strains are not expected to be more clinically virulent since *S. aureus* gastroenteritis is self-limiting.

In most outbreaks, food has been contaminated after processing by food workers who are carriers of enterotoxin-producing strains of staphylococci.[51] It was once observed that many of these workers had visible lesions on the hands or nose. This is an uncommon finding now, although it is true that *S. aureus* of the same phage type as that in the contaminated food can be found in food handlers in roughly two-thirds of outbreaks.[46] These handlers are generally asymptomatic carriers.

Even though many animals and animal carcasses are contaminated before processing, this usually does not result in food poisoning. Competitive growth from other flora usually contains the slower-growing staphylococci. One exception to this is milk, which can be heavily contaminated with *S. aureus* when obtained from a mastitic animal.[22]

5.5. Pathogenesis and Immunity

5.5.1. Pathogenesis. There are three prerequisites for staphylococcal food poisoning to occur: (1) contamination of a food with enterotoxin-producing staphylococci; (2) a food that provides the necessary growth requirements for the organism; and (3) appropriate time and temperature for the organism to multiply. To date, enterotoxins A through J have been the most commonly implicated enterotoxins in outbreaks of staphylococcal food poisoning. This may change, however, as tests are developed to identify more recently discovered enterotoxins.

5.5.2. Immunity. It is not clear whether immunity develops in this illness. Although attack rates are not usually 100% in outbreaks, many individuals may not consume the enterotoxin or enough of the implicated vehicle. In outbreaks where small groups are involved in which everyone consumes the implicated food, attack rates are very high (80–100%). Early attempts to "immunize" man or develop "tolerance" to toxin were not successful when broth filtrates of toxin-producing staphylococci were administered by mouth.[52]

Clearly, individuals have differing sensitivity to enterotoxin; in one investigation using volunteers, as little as 0.5 ml of a filtrate could cause illness in one person, whereas 13 ml administered to another individual caused no symptoms. Whether this is a case of immunity or physicochemical sensitivity of a receptor site is not clear. Whether immunity from one toxin confers cross-immunity to others has not been determined.

5.6. Patterns of Host Response

5.6.1. Clinical Features. The symptoms of staphylococcal food poisoning are primarily profuse projectile vomiting, nausea, and abdominal cramps, often followed by diarrhea.[37,53–55] In severe cases, blood may be observed in the vomitus or stool.[56] Rarely, hypotension and marked prostration occur. Fatalities are unusual, and recovery is complete in 1–3 days in healthy individuals.[38,57] Fever is not a common accompaniment, but may be present if dehydration is severe.

5.6.2. Diagnosis. Staphylococcal food intoxication should be considered in anyone who presents with severe vomiting, nausea, cramps, and some diarrhea. A history of ingesting meats of high salt content may be helpful. Usually, the best epidemiological clue is the short incubation period (usually 2–4 h) and the absence of fever. Of the bacterial foodborne diseases, only *B. cereus* emetic-type illness has a similar presenting illness with a short incubation period. This disease is so closely allied with fried rice as to allow an easy distinction.

6. *Bacillus cereus*

6.1. Historical Background

One of the first outbreaks implicating an aerobic spore-forming organism was described by Lubenau[58] in 1906, in which 300 inmates of a sanitorium developed a diarrheal

illness from meatballs contaminated with an organism labeled *Bacillus peptonificans*. This isolate closely resembled what is today considered typical of *B. cereus*. During the several decades following this description, other European investigators cited similar aerobic spore-forming bacilli in food poisoning involving vanilla sauce, jellied meat dishes, and boiled beef. However, it was not until Hauge's[59] description of four separate Norwegian outbreaks in 1955 that *B. cereus* was conclusively implicated as a cause of diarrheal illness. These outbreaks, all linked to vanilla sauce containing large numbers of *B. cereus*, produced an illness characterized by profuse, watery diarrhea, abdominal pain, nausea, but rarely vomiting or fever. The incubation period averaged 10–12 h, with illness resolving within 12–24 h. Subsequent experiments in which Hauge and other volunteers ingested vanilla sauce inoculated with *B. cereus* substantiated the role of this organism.[59]

After Hauge's report, outbreaks of similar illness were described in northern and eastern Europe, particularly in the Netherlands and Hungary. The first well-documented outbreak of gastrointestinal disease in the United States was reported by Midura et al.[60] in 1970.

In 1974, another form of *B. cereus* illness was recognized that involved fried rice.[61] This illness was distinctly different from those previously described, in that the incubation period was shorter, ranging from 1 to 6 h, and the illness was primarily vomiting (although some patients had diarrhea as well). Although this condition closely resembled staphylococcal food poisoning, *B. cereus* was recovered from the fried rice.

Since this report, it has become accepted that *B. cereus* is capable of producing two distinct foodborne syndromes. The emetic illness, usually associated with rice-based dishes, is caused by ingestion of a preformed heat-stable toxin that is produced by the bacteria in the food. The diarrheal illness, associated with a large variety of foods, has a longer incubation period and is caused by at least one of two heat-labile toxins that are produced by the bacteria in the intestine.

6.2. Microbiology

6.2.1. Biological Characteristics of the Organism. *B. cereus* is a gram-positive, catalase-positive, aerobic spore-forming rod that is mobile by means of peritrichous flagella. Most strains are β-hemolytic.

Strains of *B. cereus* produce a wide variety of exotoxins, but only two categories appear to be important in the pathogenesis of food poisoning: the "diarrheogenic enterotoxin" and the "emetic toxin." Early studies of enterotoxin suggested that there was one diarrheogenic toxin, but since then, three enterotoxins have been characterized. Two enterotoxins are associated with diarrheal syndrome: the three-component hemolysin BL toxin (HBL) and the three-component nonhemolysin enterotoxin (Nhe). It is not known how much a third single component enterotoxin[62] contributes to food poisoning.[63,352] HBL is not only hemolytic, but also induces fluid accumulation in rabbit ileal loop and necrosis of villi, and forms pores in eukaryotic cell membranes.[64,65] Studies indicate that Nhe shares some sequence and structural homologies with HBL.[66] Strains and other *Bacillus* species can produce one or both of the multi-component enterotoxins. At present, it is unclear how important each toxin is to the epidemiology of food poisoning. A number of other possible enterotoxins have been reported but their biological activity has not been defined.[66]

The emetic toxin, which has in recent years been named cereulide, was first isolated from a strain implicated in an outbreak of vomiting-type illness. Cereulide is a cyclic peptide composed of three repeats of four amino acids, and has a molecular weight of 1.2 kD. In contrast to the diarrheal toxins, cereulide is highly stable, resisting heat, pH and proteolysis. It is thought to bind the 5-HT3 receptor and stimulate the vagus afferent.[67] In experiments using animal models (rhesus monkey and Asian musk shrew), the emetic dose of cereulide was found to be between 9 and 12 μg/kg.[68,69] Production of emetic toxin is linked with temperature [highest elaboration occurring between 12 and 15°C[70]] and quite possible sporulation.[71] Cases of liver failure caused by *B. cereus* food poisoning,[72,73] has led to the discovery that the emetic toxin is a mitochondrial toxin.[72]

6.2.2. Laboratory Diagnosis. *B. cereus* can be cultured on blood agar, grown aerobically at 37°C. Isolates from blood agar form flat, granular β-hemolytic colonies, resembling "ground glass". The FDA recommends use of a mannitol–egg yolk–polymyxin agar with incubation for 24–48 h at 30°C.[74] This medium utilizes mannitol (which *B. cereus* cannot catabolize), the selective agent polymixin B, and a lecithinase reaction on the egg yolk for identification of *B. cereus* isolates. Chromogenic media are also being utilized. Enumeration of *B. cereus* can be performed either by direct plating or, if expected numbers are less than 1,000/g, by most probable number methods.[74] *B. cereus* has 16S and 23S ribosomal sequences that are nearly identical to sequences in *B. thuringienisis*, *B. mycoides*, and most importantly *B. anthracis*. Need for proper differentiation of *B. cereus* from *B. anthracis* has become increasingly important in the post-September 11th era. Traditionally, tests for motility, rhizoid growth, hemolytic activity, and protein toxin crystals were used to distinguish *B. cereus* from other species.[74] However, recent experiments have indicated these tests may not be adequate.[75–77]

Animal models such as monkey feeding and rabbit ileal loop assays have been used for detection of the diarrheagenic toxin as have cytotoxic cell assays. However, the validity of these assays needs further assessment. Commercial kits have been developed that employ reverse passive latex agglutination and enzyme immunoassay methods.[66] The diarrheogenic toxin can also be demonstrated in *B. cereus* cells by PCR or plasmid profile analysis.[78] However, even when sought, the enterotoxin is often not found because of the instability of this molecule, which is rapidly degraded by proteolytic enzymes.[79] There are as yet no commercially available immunoassays for detecting emetic toxin. Typically, culture filtrates from emetic food-poisoning samples are tested using a HEp-2 vacuolation assay, a sperm-based bioassay, or LC-MS analysis.[68] Recently, a PCR assay was developed against a gene specific to emetic toxin-producing *B. cereus* strains, which allows rapid detection of bacteria during food outbreaks.[43,73]

6.3. Descriptive Epidemiology

6.3.1. Prevalence and Incidence. Because *B. cereus* food poisoning is not a reportable disease and misreporting may occur as symptoms are mistaken for those caused by *C. perfringens* (diarrheal) or *S. aureus* (emetic), the true incidence of *B. cereus* illness is unknown. As with many foodborne pathogens, disease due to this organism often goes undiagnosed and unreported, mainly due to mild symptoms and short disease duration. Outbreak surveillance data from 1990 to 2002 (Table 4) show that this pathogen accounts for 1.5% of foodborne outbreaks in the United States. The average size of these outbreaks is 24 cases.

In Europe, *B. cereus* has accounted for a larger portion of outbreaks, most of which are of the diarrheal type. In Norway, for example, 33% of all food-poisoning cases from 1988 to 1993 were due to this organism.[80] In Hungary, *B. cereus* was the third most common cause of foodborne illness from 1960 to 1968.[81] The relatively high incidence was attributed to a preference in the Hungarian population for well-spiced meat dishes. The spices used were found to contain large numbers of *Bacillus* spores. Poor postcooking storage procedures allowed the spores that survived cooking to germinate. In Japan, it is the emetic type that is reported more frequently.[81]

6.3.2. Epidemic Behavior and Contagiousness. In the original description by Hauge,[59] 82% of individuals who ate the incriminated meal were affected. Of the 19 who were not ill, 11 had not eaten the dessert (implicated vehicle), and 8 had eaten only a small quantity. Other reported outbreaks have indicated attack rates from 50 to 75%.[82,83] In the reported vomiting-type outbreaks, virtually all individuals who consumed contaminated food became ill. There is minimal risk of secondary cases, since heavy contamination of the implicated vehicle is required.

6.3.3. Geographic, Temporal, Age, Gender, Racial, and Occupational Distribution. *B. cereus* food poisoning has not shown any geographic or temporal distribution. Children and elderly may become more severely affected and require hospitalization. There does not appear to be any gender, racial, or occupational predisposition.

6.3.4. Occurrence in Different Settings. Most reports of diarrheal illness have taken place in institutional settings (schools, hospitals, and others). This may be a reporting artifact, since foodborne outbreaks are more easily recognized in confined populations.

6.3.5. Other Factors. The clear-cut association between the vomiting syndrome and fried rice deserves emphasis. The first three reports of outbreaks of emetic-type *B. cereus* illness in the United States[84] and the report by Mortimer and McCann[61] of five outbreaks in Great Britain implicated fried rice as the vehicle. This association has been attributed to the method used to prepare this food in Chinese "takeout" restaurants (see below). Other foods implicated in these types of outbreaks are pasta salad, mayonnaise, and turkey loaf, and hot chocolate from vending machines.

The diarrheal illness has also been caused by a large variety of foods including barbequed pork, boiled beef, sausage, chicken soup, macaroni and cheese, vanilla sauce, and puddings.

6.4. Mechanisms and Routes of Transmission

The frequent isolation of *B. cereus* in agricultural products is due to its abundance in soil. Because it comprises the natural flora of many foods, *B. cereus* has been found in about 25–50% of foodstuffs sampled, including cream, pudding, meat, spices, dry potatoes, dry milk, infant foods, spaghetti sauces, and rice.[85–87] Contamination of the food product generally occurs prior to cooking. If the food is prepared in such a manner that the temperature is maintained at 30–50°C, vegetative cell growth will occur. Spores can survive extreme temperatures, and when allowed to cool relatively slowly, they will germinate and multiply.[88] For these reasons it is recommended that food be maintained at 60°C or higher and if it is to be stored, cooled rapidly to below 8°C.

The association of the emetic-type illness with fried rice can be attributed to the method in which this food is prepared in Chinese restaurants. Rice is first boiled in bulk and then stored at room temperatures for periods as long as 3 days. Chefs in these institutions are usually reluctant to refrigerate the rice because this causes it to clump. The rice is then rapidly fried when needed with a mixture containing

beaten eggs. The heat produced during this second cooking is insufficient to destroy the heat-stable emetic toxin. Studies have found viable *B. cereus* in up to 93% of specimens of boiled rice, though the number of organisms is usually less than 10^5/g.[87–89]

Human fecal carriage is not believed to play a role in transmission of *B. cereus*, though this organism can transiently colonize the intestinal tract for periods of up to 4 days.[90] The isolation rate of *B. cereus* in the stool of asymptomatic individuals can be as high as 40% and is thought to be due to the high prevalence of this species in foods.[91,92] The European Food Safety Authority has even approved probiotic use of *B. cereus* strains that produce minute amounts of toxin.

6.5. Pathogenesis and Immunity

6.5.1. Pathogenesis. *B. cereus* food poisoning is toxin-mediated in both the diarrheal form and the emetic form.[84] Whether the diarrheogenic, heat-labile enterotoxin is actually ingested or produced in vivo is not known; there are, however, several pieces of evidence to suggest that the latter mechanism may be operative. The incubation period in the diarrheal illness is longer than might be expected for preformed toxin (>6 h; average 12 h), and a large inoculum (10^5–10^8 cells/g) is required to cause illness, suggesting a requirement for intestinal colonization.

The emetic illness is presumptively caused by preformed toxin that is heat stable. The short incubation period and high attack rate provide support for this pathogenic mechanism.

6.5.2. Immunity. Our knowledge of immunity to either of these syndromes is largely uncharted. Antiserum to the diarrheogenic enterotoxin introduced into the rabbit ileal loop model along with the toxin can prevent fluid accumulation; no careful studies in humans have been performed. There are many serotypes of *B. cereus*, but it is not known whether the various enterotoxins have immune cross-reactivity. The emetic toxin is poorly antigenic [when repeatedly challenged with this, rhesus monkeys develop no resistance to its action[79]] but may still have immunomodulating properties (inducing apoptosis in natural killer cells).[93]

6.6. Patterns of Host Response

6.6.1. Clinical Features. The initial report by Hauge[59] described the diarrheal illness of *B. cereus*, and his findings are consistent with what we know today. Illness begins after a 10–12 h incubation period is characterized by diarrhea (96%), abdominal cramps (75%), and vomiting (23%). Fever is uncommon. The duration of illness has ranged from 20 to 36 h, with a median of 24 h.[59,84]

The emetic form of the illness has a short incubation period that varies from 1 to 6 h and the predominant symptoms of vomiting (100%) and abdominal cramps (100%). Diarrhea is present in only one-third of affected individuals.[84] The duration of this illness has ranged from 8 to 10 h, with a median of 9 h. In both types of illness, the disease is usually mild and self-limited.

6.6.2. Diagnosis. The diagnosis of *B. cereus* food poisoning should be considered in any individual who has diarrhea without fever in association with lower abdominal cramps. The incubation period varies from 6 to 24 h. The easiest way to make the diagnosis is by culture of the incriminated food product. The disease caused by *C. perfringens* is so similar to the diarrheal form of *B. cereus* that they cannot be differentiated clinically or epidemiologically; culture methods are required. The emetic form of the disease should be suspected in an individual presenting with nausea and vomiting 1–5 h after consumption of a farinaceous food, especially fried rice. The clinical picture is indistinguishable from that of *S. aureus* food poisoning.

Because *B. cereus* can be found in many foodstuffs, this organism should only be considered significant if present in the incriminated food item in numbers of 10^5 or more per gram.[7] Similarly, since *B. cereus* can sometimes be found in the stools of healthy persons[91,92] isolation of the organism from feces may not be a suitable confirmation unless negative stool cultures are obtained from an appropriate control group. As a result, phage typing of *B. cereus* has emerged as a reliable resource in epidemiological investigations allowing one to trace isolates to implicated foods/sources.[94,95] PFGE techniques are used as well.[96] Serotyping has also proven useful in epidemiologic studies. Forty-two serotypes have been identified based on classification of spore, somatic, and flagellar antigens. Twenty-three of the 42 serotypes are associated with *B. cereus*-related disease.[66] Of 200 outbreak-related *B. cereus* isolates from 11 different countries between 1971 and 1986, 91% were typeable by this system.[81] Of these, 127 (64%) were H-type 1, a serotype found in 23% of cooked rice. H-1 has been positively correlated with production of emetic toxin,[67,81,97] along with types 5, 8, 12, and 19.[66] The flagellar (H) antigens most commonly associated with diarrheal food poisoning are 1, 2, 6, 8, 10, 12, and 19.[66]

7. *Clostridium perfringens*

7.1. Historical Background

C. perfringens was first recognized and confirmed in the United States as a foodborne pathogen in 1945 by

McClung,[98] who studied four outbreaks of diarrhea related to the consumption of chickens steamed 24 h prior to consumption. *C. perfringens* was isolated from the cooked chickens. Prior to this discovery, workers in Europe had also recognized that gravy contaminated with anaerobic spore-forming bacilli, including *C. perfringens*, had caused gastrointestinal illness in children.[98,99]

Shortly after McClung's discovery, filtrates from strains of *C. perfringens* were administered by mouth to human subjects.[100] Cramps and diarrhea occurred in some individuals, but the incubation period was short: 45–80 min. Living cultures induced cramps and bloating in 4 h and diarrhea several hours thereafter. Hobbs et al.[101] elegantly confirmed these results and outlined the epidemiological features of the disease in Great Britain in 1953.

The other major discovery in the late 1940s was the outbreak of a severe and often lethal intestinal disease-labeled enteritis necroticans or "Darmbrand" that affected over 400 people in Germany;[102] this outbreak was similar to others described later in New Guinea and termed "pig bel"[103] (see Section 7.7). Both conditions were due to *C. perfringens*. The strains were originally designated *Clostridium welchii* type F but were later reclassified as *C. perfringens* type C.

Up to this time, all foodborne outbreaks were felt to be due to heat-resistant *C. perfringens*. Several investigators in the late 1960s confirmed that nonhemolytic and β-hemolytic, heat-sensitive strains could cause food poisoning.[104]

Investigations in the late 1960s and early 1970s provided convincing evidence that the pathogenesis of *C. perfringens* food poisoning is due to enterotoxin production while cells are sporulating. Furthermore, enterotoxin can be demonstrated in *C. perfringens* types A, C, and D. The toxins of types A and C are quite similar with regard to physicochemical properties.

7.2. Microbiology

7.2.1. Biological Characteristics of the Organism. Clostridia are gram-positive, spore-forming anaerobes. Although all species grow better under anaerobic conditions, *C. perfringens* is remarkably aerotolerant and may survive exposure to oxygen for as long as 72 h. This organism has a characteristic "boxcar" appearance on Gram-stained slides and, unlike most *Clostridium* species, is nonmotile. The spores produced by this organism are oval, subterminal, and only rarely seen in clinical specimens.

C. perfringens produces at least 15 toxins that contribute to its virulence. The species is divided into five types, A–E, on the basis of four major toxins, α, β, ε, and ι and enterotoxin genes. Type A is the agent of classic *C. perfringens* food poisoning. Almost all strains of this type isolated from outbreaks produce α-toxin (a lecithinase).[105,106]

The best characterized enterotoxin identified thus far is CPE, a heat-labile protein with a molecular weight of approximately 35 kD. CPE is produced by type A isolates. A review by McClane suggests that CPE is elaborated in the cytoplasm of *C. perfringens* during its sporulation in the intestine.[107] Lysis of *C. perfringens* at the end of sporulation releases CPE into the intestinal lumen, where it binds receptors on the surface of intestinal epithelial cells.[107] CPE is thought to be pore-forming, subsequently altering membrane permeability and compromising tight junction structure and function.[107] The role of this toxin in producing diarrheal illness was confirmed by feeding cell-free filtrates to volunteers. Of 15 persons who consumed filtrates from toxin-producing strains, four developed diarrhea, whereas none of 16 persons fed filtrates from toxin-negative strains developed illness. The incubation period for illness in these volunteers was 2–2.5 h. Similar results have been obtained in experimental animals.[108,109] Another type A enterotoxin (θ-toxin) has been discovered to induce food-related diarrhea in humans and animals.[110] The significance of this pore-forming cytolysin is questionable, however *C. perfringens* strains with inactivated *cpe* genes were unable to affect rabbit ileal loops,[111] suggesting that CPE is required for induction of diarrheal symptoms. A β2-toxin (CPB2) has been thought to have diarrheagenic properties due to its high prevalence in diarrheic piglets.[112] Although β2-toxin has not been associated with human illness yet, it has been found produced by type A *C. perfringens* gastrointestinal disease isolates containing the *cpe* gene on plasmids.[113]

C. perfringens is ubiquitous in the environment. It can be found in almost any soil, where it performs its principal role in nature: the breakdown of organic waste. A 2004 study found that 1.4% of 900 samples of non-outbreak American retail foods are contaminated with *cpe* gene-containing *C. perfringens* isolates.[114] However, another study found no *cpe*-positive *C. perfringens* isolates in 131 retail samples.[115] Both *cpe*-positive and *cpe*-negative organisms are also a common component of the gastrointestinal tract of healthy humans and animals; surveys of asymptomatic individuals show that it can be isolated from almost any stool sample if enrichment techniques are used, but it is usually present at concentrations less than 10^4 CFU/g, except in the elderly, where it frequently exceeds 10^6 CFU/g. The lowest numbers are found in vegetarians, which may indicate that animals are a source of pathogenic *C. perfringens* strains. Because this organism is part of the human natural flora and isolation is complicated by the presence of a large number of *cpe* gene-negative cells,[116–118] enumeration of *cpe* gene-positive spores in the feces and suspected food is recom-

mended when attempting to confirm *C. perfringens* as the cause of a foodborne outbreak.[119] Interestingly, the *cpe* gene has been found not only on the chromosome of *C. perfringens* food-poisoning isolates, but on the plasmid of non-foodborne gastroenteritis-causing isolates.[120,121] Although there are exceptions to this observation,[122,123] it has been useful in epidemiological investigations.

7.2.2. Laboratory Diagnosis. The selection of media is dependent on whether the sample is fecal or food. The FDA-recommended culture medium is the egg yolk–free tryptose–sulfite–cycloserine agar.[124] It allows sulfite-reducing clostridia to form black colonies and inhibits other facultative anaerobes.[125,126] Colonies can then be selected for nitrate reduction, non-motility, liquefaction of gelatin, and glucose fermentation.[125]

Three other characteristics are of use in the isolation of *C. perfringens*: a dual zone pattern of hemolysis on blood agar plates, opalescence (precipitation) on egg-yolk media, and "stormy fermentation" in milk media. On blood agar, *C. perfringens* colonies are surrounded by a narrow inner ring of incomplete hemolysis due to θ-toxin, and a much wider outer ring of complete hemolysis due to α-toxin. On egg-yolk agar, colonies produce opalescence, termed the "Nagler reaction," which is due to the presence of α-toxin. Presumptive identification of *C. perfringens* can be achieved with an egg-yolk agar plate, which is partly coated with *C. perfringens* α-antitoxin, which inhibits the opalescence. Stormy fermentation in milk media is characteristic of *C. perfringens*. Vigorous lactose fermentation produces both acid, which coagulates the casein in the milk medium, and large amounts of gas, which breaks apart the clot.[127] For enumeration, tryptose–sulfite–cycloderine (TSC) agar is recommended.[128]

None of these reactions are 100% sensitive or specific for *C. perfringens*. Other *Clostridium* species produce lecithinases that are inhibited by the α-antitoxin. Similarly, other species ferment lactose rapidly and produce stormy fermentation. Furthermore, many outbreak-related strains do not produce a double zone of hemolysis. Several lecithinase-negative strains have been isolated from outbreaks in the United Kingdom.[129]

Most enrichment techniques do not distinguish between causative and non-causative strains, and therefore molecular epidemiologic methods are recommended during outbreak investigations.[130] Labs with readily available molecular technology have used PCR of the *cpe* gene.[131] The observation that food-poisoning isolates carry a chromosomal *cpe* gene, while non-foodborne gastrointestinal (GI) disease isolates have a plasmid *cpe* gene has motivated the use of a multiplex PCR assay.[132] PFGE with *Sma*I and *Apa*I enzymes has also been used.[117,133] However, other studies have shown that enterotoxigenic data must accompany PFGE in order to increase the validity of etiologic analysis.[116] Fecal enterotoxins can be detected by ELISA,[134] counterimmunoelectrophoresis, and reversed passive latex agglutination assays.[116,135,136] DNA probes,[137] ribotyping,[118] and plasmid prophiles[138,139] can also be used to test enterotoxigenicity of strains of *C. perfringens* isolated from feces and food during outbreaks. A newly developed method, hydrophobic grid membrane filter-colony hybridization (HGMF-CH), can be used for enumeration and *cpe* detection using both biochemical and molecular hybridization techniques.[119]

7.3. Descriptive Epidemiology

7.3.1. Prevalence and Incidence. Almost all of our knowledge of the epidemiology of *C. perfringens* food poisoning comes from the study of outbreaks. Table 4 summarizes these data for the United States from 1990 to 2002. Given that the diagnosis of *C. perfringens* food poisoning is difficult to confirm because of the ubiquity of the organism in foods and stools, it is likely that a *C. perfringens* also accounts for at least some of the foodborne outbreaks for which an etiology is not found.

Sporadic *C. perfringens* food poisoning is even less likely to be recognized, since definitive diagnosis in this setting is very difficult. Further developments in enterotoxin detection assays are likely to change this.

7.3.2. Epidemic Behavior and Contagiousness. Epidemics of *C. perfringens* are usually characterized by high attack rates (usually >50%) with a large number of affected individuals; there were 62 cases per outbreak for the years 1990–2002 (see Table 4). There is no risk of secondary transmission.

The incubation period in most outbreaks varies between 8 and 14 h (median 12 h), but can be as short as 6 h or as long as 24 h.

7.3.3. Geographic, Age, Gender, Racial, and Occupational Distribution. Since the organism is widespread in nature, there is no geographic clustering associated with this organism. Outbreaks have been recorded worldwide. No age, gender, racial, or occupational predilections are known.

7.3.4. Temporal Distribution. It has been stated for many years that outbreaks of *C. perfringens* are more common in the spring and fall in contrast to most other foodborne pathogens, which peak during the summer. However, no such trend has been noted in the United States.

7.3.5. Occurrence in Different Settings. Outbreaks due to this organism are most frequently reported from institutions or large gatherings. The pathogenesis of infection

requires that a meat or fish dish be precooked, slow-cooled, and then reheated to be served. The association with large gatherings may be due to the practice of preparing food in advance for such events or may be simply a reporting artifact, since only such groups may recognize the outbreak as food poisoning.

7.3.6. Nutritional Factors. Proteinaceous meats such as beef, turkey, and chicken are the most frequent vehicles of infection with *C. perfringens*; other foods implicated include other animal and vegetable protein sources such as gravy, stew, minestrone soup, bean burritos, rice pudding, and fried fish paste. *C. perfringens* is common in many foods: It can be recovered from 30 to 80% of raw or frozen meat and poultry,[140] though most strains found in food are nonenterotoxigenic.[141] Cured meats are rarely vehicles in outbreaks as the growth of *C. perfringens* is sensitive to high salt mediums.

7.4. Mechanisms and Routes of Transmission

Most outbreaks involve meat-based meals that have been precooked in bulk, allowed to cool for several hours, and then inadequately reheated. The initial cooking is generally sufficient to kill the vegetative organisms but not the spores, which germinate when the temperature falls below 50°C. Unless this food is reheated to a temperature greater than 75°C, it will contain many viable organisms.[142] In most outbreaks of *C. perfringens* food poisoning, the original source of the bacteria has been assumed to be the food itself.

7.5. Pathogenesis and Immunity

7.5.1. Pathogenesis. Symptoms in *C. perfringens* food poisoning are caused by in vivo production rather than by ingestion of preformed enterotoxin.[108,109,141] In volunteer experiments, this toxin is capable of producing the symptoms of food poisoning, but only when given in much higher amounts than found in epidemiologically linked foods. In fact, by the time enough enterotoxin has accumulated in food to be detectable, the food is generally unpalatable.[143] The fact that the toxin is produced by the bacteria in the intestines accounts for a longer incubation period than that seen in illness such as *S. aureus* food poisoning, which is due to preformed toxin.

Relatively large numbers of organisms are required to produce illness. An early study showed that ingestion of 10^8 organisms was capable of producing illness in 50% of volunteers.[144] Others have estimated that five times that number may be necessary.

Once ingested, the organism proliferates in the intestine, sporulates, and produces its enterotoxin. The toxin binds to a receptor on the brush border of the intestine and inserts into the membrane where it remains attached to the external membrane surface.[145–147] By doing this, the toxin increases permeability of the membrane to ions which leads to salt and water secretion into the lumen and increased intestinal motility.[148] In addition, the clostridial toxin inhibits glucose transport, damages the intestinal epithelium, and causes protein loss into the intestinal lumen. These effects are most prominent in the terminal ileum.[149,150] *C. perfringens* enterotoxin has also been shown to act as a superantigen, stimulating proliferation of T cells.[151]

7.5.2. Immunity. Immunity is not well understood. In one study, 65% of Americans and 84% of Brazilians had antienterotoxin activity in serum.[152] The significance of this finding is unknown. No outbreaks have been studied by these methods in which blood was available prior to the outbreaks so that antienterotoxin immunity could be assessed. In animal studies, enterotoxin antiserum can block the action of the toxin on ligated rabbit loops. It has been suggested, however, that the presence of antibody in serum has little or no effect on toxin activity in the intestine.[153,154]

7.6. Patterns of Host Response

7.6.1. Clinical Features. *C. perfringens* food poisoning is characterized by watery diarrhea and severe, crampy abdominal pain, usually without vomiting, beginning 8–24 h after the incriminated meal. Fever, chills, headache, or other signs of infection usually are not present.

The illness is of short duration, 24 h or less. Rare fatalities have been recorded in debilitated or hospitalized patients who are victims of clostridial food poisoning.

7.6.2. Diagnosis. The diagnosis of *C. perfringens* food poisoning should be considered in any diarrheal illness characterized by abdominal pain and moderate to severe diarrhea, *unaccompanied* by fever or chills. There are usually many other individuals involved in the outbreak, and the suspect food is beef or chicken that has been stewed, roasted, or boiled earlier and then allowed to sit without proper refrigeration. Diagnosis should be made based on detection of 10^5 *C. perfringens*/g in the food sample, and detection of at least 10^6 *C. perfringens* spores/g of fecal material.[155]

B. cereus food poisoning can have a very similar presentation and can be ruled out only by bacteriological study. Enterotoxigenic *E. coli* can also present in this fashion (see Chapter 15), although low-grade fever may be present.

Cholera has more profuse diarrhea, which helps differentiate it from clostridial intoxication. *Salmonella* infection has a longer incubation period and is sometimes accompanied by fever (Chapter 32). CDC criteria for confirmation of an outbreak of *C. perfringens* food poisoning are shown in Table 3. It should be noted when testing implicated foods that numbers of viable clostridia decline rapidly in refrigerated samples.

Several subtyping methods are of use in epidemiological studies. Serotyping has proven useful in the United Kingdom but has been less successful in the United States, where many strains are untypeable with existing reagents. Pulsed-field gel electrophoresis methods have also proven useful for molecular subtyping of *C. perfringens* isolates.[133] It is often advised to test more than one isolate from a fecal sample, since carriage of *C. perfringens* in the human gastrointestinal tract is normal. Bacteriocin typing,[156] plasmid typing,[157] and ribotyping[118,158] have also been successfully applied to the investigations of *C. perfringens* outbreaks.

7.7. Enteritis Necroticans

Enteritis necroticans deserves to be considered separately from type A food poisoning because of its unique clinical features, epidemiology, and pathobiology. This illness is a much more severe, necrotizing disease of the small intestine with a high mortality. The incubation period is 24 h, after which illness ensues with intense abdominal pain, bloody diarrhea, vomiting, and shock. The mortality rate is about 40% and is usually due to intestinal perforation.

In postwar Germany, where the illness was known as Darmbrand ("burnt intestine" or "fire belly"), illness was linked to the consumption of underprocessed canned meat that contained *C. perfringens* type C spores. Women were disproportionately affected, particularly those aged 30–60 years. In 1947, the incidence was 11 in 100,000 and cases peaked in the summer.[102]

In Papua New Guinea, outbreaks of "pig-bel" have been clearly related to the consumption of pork during prolonged feasts. During such feasts, the pig is often improperly cooked, and large quantities of pork are consumed over 3 or 4 days. There is a clear male preponderance of cases, with attack rates being highest in boys less than 10 years of age. The incidence of pig-bel was as high as 500 in 100,000 on the island prior to the introduction of the β-toxoid vaccine (see below).[159]

Sporadic cases of enteritis necroticans have been described in several other countries, particularly in the developing world.[160] An outbreak of this illness occurred in a Khmer refugee camp in Thailand between 1985 and 1986; the incidence reached 440 in 100,000 in the 0- to 10-year-old age group, with a case-fatality rate of 58%.[160]

The organism that causes enteritis necroticans is typically *C. perfringens* type C, which does not generally produce enterotoxin. β-toxin is implicated in this disease. This toxin is a 48 kD protein that is extremely sensitive to proteases. It produces hemorrhagic necrosis of the small intestine, especially at the level of the jejunum. But ingestion of β-toxin-producing bacteria is not sufficient in itself to produce enteritis necroticans. At least three other factors play a role in the pathogenesis of this disease: a low-protein diet, the presence of protease inhibitors, and reduced gastric activity. All of these factors have the effect of decreasing the amount of trypsin in the intestine, and thereby permitting the toxin to act on the intestinal wall.[161,162]

Thus, in postwar Germany, epidemic malnutrition was an important enabling factor. In Papua New Guinea, the usual low-protein diet coupled with trypsin inhibitors in sweet potatoes (commonly eaten during pig feasts) or secreted by ascaris (present in 70–80% of the highland population) have been identified as factors in the pathogenesis of pig-bel.[103]

Antibodies to β-toxin are protective against enteritis necroticans. These antibodies, which are largely absent in European adults and Papuan children, are highly prevalent in Papuan adults; these antibodies may account for the lower incidence of pig-bel in the adult population of the island. A β-toxoid vaccine has been developed and is protective against this disease. The incidence of pig-bel in Papua New Guinea has declined dramatically since the introduction of this vaccine in the early 1980s.[103] More recent cases of necrotizing enterocolitis in previously healthy adults have suggested that *C. perfringens* type A may also cause this disease.[163,164]

8. "Noncholera" *Vibrio* Species

8.1. Historical Background

In the 1930s, cholera researchers realized that *Vibrio cholerae* isolates could be divided into two groups: those that agglutinated with serum from cholera patients (0 group 1) and those that failed to agglutinate. This latter group consisted primarily of environmental isolates that were thought to be nonpathogenic, although there was early speculation that some of these "nonagglutinating" or "noncholera" vibrios might be responsible for such syndromes as "paracholera." Firm evidence of the pathogenicity of these

strains did not emerge until two decades later when the first outbreaks due to these organisms were identified and investigated. As these data accumulated, it became increasingly clear that many of the strains were so distinct from *V. cholerae* as to constitute separate species.[165]

Within the species *V. cholerae*, there are over 150 O groups, only two of which cause epidemic cholera: *V. cholerae* O1 and *V. cholerae* O139. Serotype O1 held a monopoly on this disease until 1992, when O139 emerged and rapidly spread in Asia (see Chapter 12). Other serotypes of *V. cholerae* ("nonepidemic" *V. cholera*) are different in that they generally cause only sporadic illness, rather than large-scale epidemics such as those for which O1 and O139 are infamous.

V. parahaemolyticus was the first noncholera *Vibrio* species to be recognized as an agent of foodborne illness. Fujino and coworkers[166] first isolated this organism from autopsy materials collected during an investigation of a food-poisoning outbreak. Over the subsequent decade, many other outbreaks in Japan incriminated a halophilic, hemolytic gram-negative organism similar to that described by Fujino. The vehicles in these outbreaks were usually raw fish, shellfish, and cucumbers in brine. The names *Pasteurella parahaemolytica, Pseudomonas enteritis*, and *Oceanomonas parahaemolytica* were all suggested before taxonomic studies firmly placed the organism within the genus *Vibrio*.

In addition to *V. parahaemolyticus*, several other *Vibrio* species are known to be enteropathogenic (see Table 6).

Table 6. Clinical Syndromes Due to *Vibrio* Species[a]

	Clinical presentation[b]		
	Gastroenteritis	Wound/ear infection	Primary septicemia
V. cholerae			
O1, O139	++	(+)	
Nonepidemic	++	+	+
V. mimicus	++	+	
V. parahaemolyticus	++	+	(+)
V. fluvialis	++	+	
V. furnissii	++		
V. hollisae	++	+	(+)
V. vulnificus	+	++	++
V. alginolyticus		++	
V. damsela		++	
V. cincinnatiensis			(+)
V. carchariae		(+)	
V. metschnikovii	(+)		(+)

[a] Adopted from Morris.[187]
[b] ++, denotes the most common presentation; +, other clinical presentations; and (+) very rare presentations.

V. fluvialis, V. furnissii, V. hollisae, and *V. minicus* have been well described as agents of sporadic gastroenteritis or of foodborne outbreaks. *V. vulnificus* can cause a syndrome of "primary septicemia" (septicemia without any other obvious source) associated with ingestion of the organism in shellfish. A Gulf Coast Vibrio Surveillance summary from 1988 to 1996 found that a majority of reported vulnificus infections were wound infections (45%) and primary septicemia (43%).[167] Numerous other noncholera *Vibrio* species (there are over 5 dozen known *Vibrio* species) have been described but are not known to be pathogenic.[168]

8.2. Microbiology

8.2.1. Biological Characteristics of the Organisms. Vibrios are ubiquitous of microbes in aquatic environments. They are most common in marine and estuarine environments, though some species such as *V. cholerae* have been isolated from fresh water. In temperate climates, their numbers vary seasonally: In the summer they can easily be isolated from water, suspended particulate matter, plankton, algae, sediment, fish, and shellfish; during the winter months, they decline markedly in number and are found primarily in sediments.[169,170] Nonepidemic *V. cholerae* has been isolated from the Chesapeake Bay, with greater detection in areas above 19°C and salinities between 2 and 14 ppt. Furthermore, modeling methods demonstrate that temperature and salinity conditions can predict the presence of *V. cholerae* with an accuracy of 75.5–88.5%.[171] Other studies in the Chesapeake Bay area have shown that *V. vulnificus* constituted 12% of the total aerobic bacterial flora present in bay water, with peak counts in the range of 10^1–10^3/ml. Counts may be two orders of magnitude higher in filter-feeding mollusks, such as oysters. Virtually 100% of raw oysters harvested during warmer, summer months carry *V. vulnificus*. Studies conducted by the FDA have identified nonepidemic *V. cholerae*, in 14% of oyster shell stock sampled.[172] Environmental isolation rates of 9.6–12.8% have been reported for *V. parahaemolyticus* in the United States.[173]

All *Vibrio* species are facultatively anaerobic, asporogenous, pleomorphic gram-negative rods. Most are highly motile with a single, polar flagellum and are oxidase positive on standard biochemical panels. One consequence of their marine habitat is that most vibrios are "halophilic," requiring salt (at least 1% NaCl) for growth; some will grow in salt concentrations as high as 12%. *V. cholerae* and *V. mimicus* are notable exceptions: Although they grow well in 1% NaCl and can tolerate salt concentrations as high as 6%, they are also able to grow in the absence of salt.

8.2.2. Laboratory Diagnosis. *Vibrio* species will grow on or in most nonselective culture media, including blood agar and commercially available blood culture media. For isolation from stool, use of a selective medium, thiosulfate, citrate, bile salts, and sucrose (TCBS agar), is generally necessary.[169,174] With the exception of some strains of *V. hollisae*, all pathogenic vibrios will grow on this medium, producing colonies that are yellow (sucrose fermenters such as *V. cholerae*) or blue-green (nonsucrose fermenters such as *V. parahaemolyticus* and most *V. vulnificus*). Because the routine use of TCBS is not considered cost-effective in the United States, its use must be specifically requested. Those labs that do not use TCBS typically screen nonselective blood plates for oxidase activity. Susceptibility to vibriostatic agent, O/129, is useful in differentiating most vibrios from other gram-negative bacteria, however, this is not always the case. Some isolates have shown resistance to O/129.[175]

Confirmation of the genus and identification of the species can be accomplished using conventional tests. Because many vibrios are halophilic, the addition of 1% NaCl to growth media is often necessary to prevent false-negative reactions. When examining environmental samples, especially water samples, concentration procedures or selective enrichment broths may be needed.[174] Direct identification from food and water samples may be facilitated by the use of specific DNA probes, PCR, and PFGE profile techniques.[176,177] In recent years, chromogenic mediums and commercial biochemical test kits have been developed to differentiate *vibrio* species.

8.3. Descriptive Epidemiology

8.3.1. Prevalence and Incidence. Because TCBS media is not routinely used by most medical microbiology laboratories, *Vibrio* infections are probably underdiagnosed in the United States. According to a FoodNet lab survey only 50% of on-site labs routinely tested for vibrio and only 27% of those labs used TCBS agar.[178] FoodNet along with the CDC Vibrio Surveillance system, started in 1988, have provided the most comprehensive data to date on the epidemiology of *Vibrio* infections in North America (see Table 7). Among 2003 FoodNet sites, the incidence of *Vibrio* spp. infections varied from 0.1 to 0.6 per 100,000 and was usually highest in coastal states, especially California and Maryland (Table 5). According to a 1996–2002 FoodNet *Vibrio* surveillance summary, *V. parahaemolyticus* was the most common laboratory-confirmed etiology (13%) followed by *V. vulnificus* and *V. cholerae* (both 8%). *V. fluvialis, V. alginolyticus*, and other vibrio species were identified less often (≤6%).[179] A global increase in *Vibrio* infections over the past 10 years is thought to be the result of many factors including an increase in the consumption of raw and lightly cooked seafood, global warming, and the emergence of more virulent vibrio serotypes.

8.3.2. Epidemic Behavior and Contagiousness. Nonepidemic *V. cholerae*, unlike *V. cholerae* O1 and O139, usually causes only sporadic illness rather than widespread epidemics. *V. parahaemolyticus*, on the other hand, frequently occurs in outbreaks. In Japan, where consumption

Table 7. Patients with Illness Associated with *Vibrio* Species Reported from Gulf Coast States in 2005[a,b]

	Complications			Site of isolation				
	Patients	Hospitalized	Deaths	Isolates	Stool	Blood	Wound	Other[c]
V. alginolyticus	40 (12)	9/37 (24)	0/37 (0)	40 (12)	2	1	25	12
V. cholerae (non- toxigenic)	33 (10)	14/31 (45)	2/28 (7)	35 (10)	19	10	4	2
V. damsela	4 (1)	3/4 (75)	0/4 (0)	4 (1)	0	0	3	1
V. fluvialis	17 (5)	8/17 (47)	1/17 (6)	17 (5)	12	2	2	1
V. furnissi	2 (0.6)	2/2 (100)	1/2 (50)	2 (0.6)	1	1	0	0
V. hollisae	4 (1)	3/4 (75)	0/4 (0)	4 (1)	4	0	0	0
V. mimicus	6 (2)	3/6 (50)	1/6 (17)	6 (2)	3	0	2	1
V. parahaemolyticus	168 (51)	29/155 (19)	0/155 (0)	168 (50)	130	3	22	13
V. vulnificus	36 (11)	31/35 (89)	10/32 (31)	37 (11)	1	23	11	2
Other	1 (.3)	0/1 (0)	0/1 (0)	2 (1)	1	0	0	0
Species not identified	10 (3)	3/9 (33)	0/9 (0)	10 (3)	6	1	1	2
Multiple species isolated	6 (2)	4/6 (67)	1/6 (17)	13 (4)	8	3	0	2
Total	327 (100)	109 (307)	16 /300 (5)	338 (100)	187	44	70	37

[a] Adopted from Vibrio Surveillance System Summary Data 2005.[346]
[b] Data are numbers; percentages are given in parentheses.
[c] Includes ear, gall bladder, peritoneal fluid, sputum, urine, and unknown source.

of raw seafood is relatively common, this organism accounts for 25–60% of all foodborne outbreaks. In the United States, where foodborne outbreaks are usually attributed to raw oyster consumption or the mishandling of seafood, 40 outbreaks involving 920 cases occurred from 1990 to 2002 (see Table 4). Secondary cases after outbreaks of *V. parahaemolyticus* appear to be uncommon. In 1996, a unique serotype O3:K6 was discovered in India and attributed to an increase in *V. parahaemolyticus* infections in that area.[180] Over the past 13 years O3:K6 has emerged as the dominant pandemic serotype in *V. parahaemolyticus* infections worldwide, spreading to other areas of Asia, Chile, the United States, Europe, and Africa. Subsequently this clone has diversified into other serotypes, O1:KUT, O4:K68, and O1:K25. These derivatives represent 28% of *V. parahaemolyticus* serotypes with pandemic potential.[181] Multi-locus sequence typing (MLST) data has provided evidence that post 1995 pandemic strains are clonally derived from serotype O3:K6 and that strains within this group may acquire other serotypes.[182]

Gastrointestinal infections with other *Vibrio* species have a much lower incidence, generally occurring as sporadic cases.[183] One large-scale outbreak of *V. fluvialis* occurred in India in 1981.[184] *V. vulnificus* incidence in the general population is low (0.6 per million) even though it accounts for 95% of seafood consumption-related deaths.[185]

The incubation period for nonepidemic *V. cholerae* in the few documented outbreaks has been from 12 h to 5 days.[186] After experimental infection with one strain of this bacterium, volunteers developed gastroenteritis in a median of 10 h (range, 5.5–96 h). The incubation period for *V. parahaemolyticus* is similar, varying from 4 to 30 h, with most illness occurring 12–24 h after exposure.

8.3.3. Geographic and Temporal Distribution. As noted above, vibrios are common environmental isolates throughout the world; however, strain isolation can vary from region to region. For instance, nonepidemic strains of *V. cholerae* are more commonly isolated from Gulf Coast regions of the United States than epidemic *V. cholerae.*[187] Patterns of illness vary as well. In Taiwan, *V. parahaemolyticus* accounted for approximately 86% of all foodborne outbreaks between 1995 and 2001.[188] Dietary habits (i.e., the consumption of raw seafood) undoubtedly play a role here. In the United States, where *Vibrio* infections are much less common, outbreaks and sporadic cases are concentrated along the Atlantic, Gulf, and Pacific coastlines. Raw oyster consumption is the most common risk factor.[167,189] In developing countries, where *Vibrio* infections are more likely to go undetected, seafood consumption is less commonly implicated as a risk factor for infection. In Cancun, Mexico, where a 2-month survey found nonepidemic *V. cholerae* in 16% of 134 stool samples obtained from persons with diarrhea, home-prepared gelatin was the only statistically significant risk factor in a case–control study.[190] Environmental investigation suggested other possible sources of infection: The organism was obtained from 86% of untreated well water samples, 92% of sewage samples, and 21% of seafood samples. A waterborne outbreak of nonepidemic *V. cholerae* occurred in Sudan in 1968, due to contamination of surface water next to a well.[191]

The incidence of noncholera *Vibrio* infections varies with season, being highest in the summer and fall. This is most likely a consequence of the higher number of vibrios in aquatic environments as well as in fish and shellfish harvested during these months.

According to the World Health Organization (WHO), the risk of *Vibrio* transmission with imported foods from endemic areas is very small. Commercial imports have not been implicated in any known *Vibrio* outbreaks. WHO recommends that importing countries agree with food exporters, to enforce good hygienic practices during food handling and processing to prevent, eliminate, or minimize the risk of any potential contamination.[192]

8.3.4. Age, Gender, Race, and Other Host Factors. Host factors such as race appear to play little role in the acquisition of gastroenteritis from *Vibrio* species. Factors such as gender or age do appear to correlate with disease. The median age of persons diagnosed with *Vibrio* gastroenteritis varies with geographic location: In Cancun, Mexico, for example, children less than 1 year of age were at highest risk, whereas in Bangladesh and Thailand cases have tended to occur in older children and adults.[190,193,194] Surveys of sporadic *V. parahaemolyticus* and *V. vulnificus* infections in the United States found a median age of 36 years (V.p.) and 35 years (V.v.) for gastroenteritis and 46 years (V.p.) and 54 years (V.v.) for septicemia.[167,195] These findings may reflect the older age of the population that consumes raw oysters. An estimated one in ten Americans occasionally eat raw shellfish[195,196] and have pre-existing medical conditions which lead to cases of sepsis. *Vibrio* also appears to be more prevalent in males than in females. A 2003 CDC *Vibrio* FoodNet survey reported that *Vibrio* incidence was 52% higher in males than females.[197] While this difference might be a reflection of gender-related food preferences, it may also be the result of an estrogen-driving mechanism.[198]

Host factors play a major role in the epidemiology of sepsis due to *Vibrio* species. Most patients with sepsis due to *V. vulnificus* and nonepidemic *V. cholerae*, for example, have an underlying chronic medical illness. Those with liver disease, such as alcoholic cirrhosis or hemochromatosis, are especially at risk. Although it has never been shown con-

clusively that sepsis infections are acquired through the gastrointestinal tract, their strong association with raw oyster consumption has always been cited as evidence to support this assertion.[165]

8.4. Mechanisms and Routes of Transmission

Foodborne transmission has been by far the most important route of transmission of noncholera vibrios in studies of epidemic and sporadic illness. As noted above, waterborne outbreaks have occurred, but are rare and are limited to the developing world. Person-to-person transmission may occur, but evidence for this is scant.

In the United States and Japan, noncholera *Vibrio* gastroenteritis is acquired almost exclusively via the foodborne route. Seafood, especially raw seafood, is the most common vehicle implicated in outbreaks and in sporadic disease. A *Vibrio* Working Group study found that 83% of patients with gastroenteritis and 96% of patients with primary septicemia had ingested raw oysters during the week prior to their illness.[167] A summary of *V. parahaemolyticus* infections in the United States between 1988 and 1997, found that 88% of those with gastroenteritis reported eating raw oysters before illness.[195] Other seafood such as crayfish,[199] clams, crabs, and shrimp have been implicated in outbreaks in the United States. In Japan, most outbreaks of *V. parahaemolyticus* have been due to saltwater fish or foods contaminated by seawater.[200]

Food-handling practices frequently play a role in outbreaks of noncholera vibrios. Seafood naturally contaminated with vibrios and held at an improper temperature can become heavily contaminated. *V. parahaemolyticus*, for example, has a generation time of 12 min under optimal circumstances and can increase its numbers by 1,000-fold in 2 h.[201] Likewise, already contaminated seafood may be cooked to an inadequate time–temperature combination sufficient to kill the organism. Finally, even uncontaminated or properly cooked seafood may be washed with contaminated salt water after preparation. The latter mechanism has been described in several outbreaks of *V. parahaemolyticus* abroad cruise ships.[202]

Because secondary transmission after outbreaks of noncholera vibrios is unusual, person-to-person transmission is thought not to be important in the epidemiology of these organisms. Long-term carriage has not been documented, though asymptomatic infections may be relatively common. From a cohort of 479 physicians attending a meeting in New Orleans who agreed to submit stool samples, 51 stool samples were positive for noncholera vibrios (*V. parahaemolyticus*, nonepidemic *V. cholerae, V. vulnificus, V. fluvialis*, and *V. mimicus*), of which 36 had been collected from individuals reporting no recent diarrheal illness.[203] In a large study conducted in Iran in 1971, 1.6% of pilgrims returning from Mecca were estimated to be carriers of nonepidemic *V. cholerae* (compared with 0.02% of persons leaving for Mecca). Isolations of this organism from stool cultures obtained from the contacts of these pilgrims increased over the following 6 months and decreased thereafter. Of these contacts, 60% reported diarrheal illness.[204]

8.5. Pathogenesis and Immunity

8.5.1. Pathogenesis

8.5.1.1. Nonepidemic V. cholerae. Given the relatively low incidence of this infection and the relatively high prevalence of these organisms in foods such as oysters, it is likely that virulence factors play a role in the epidemiology of these infections. Three enterotoxins—cholera toxin (CT), NAG-ST, and E1 Tor hemolysin—have been described. The 85 kD heat-labile cholera toxin is thought to bind to specific receptors in the lumen, resulting in massive loss of liquid in the intestine; additional secretory mechanisms are unclear. CT-producing species have been isolated most commonly in the Indian subcontinent and are associated with more severe nonepidemic *V. cholerae* disease.[205] However, these CT-producing strains represent only a small proportion of nonepidemic *V. cholerae* cases.[206,207] Non-agglutinable *Vibrio cholerae* heat-stable enterotoxin (NAG-ST) is similar to heat-stable enterotoxins found in *V. parahaemolyticus*, *E. coli*, and *Yersinia enterocolitica*. Its proposed mechanism is stimulation of nitric oxide production in intestinal cells.[208] A NAG-ST-producing strain has been demonstrated to cause diarrhea in young, previously healthy volunteers, whereas an otherwise similar but NAG-ST-negative strain was unable to do so despite being able to colonize the gastrointestinal tract.[209] As with CT-producing strains, NAG-ST-positive strains account for only a small portion of all clinical nonepidemic *V. cholerae* isolates.[210,211] E1 Tor hemolysin (aka *V. cholerae* cytolysin (VCC) or HlyA), on the other hand, is present in all nonepidemic *V. cholerae* strains and is responsible for the hemolytic activity of this species. More recently a novel hemolysin has been identified in a *V. cholerae* strain, although it is thought to not contribute to pathogenicity.[212] El Tor hemolysin has demonstrable enterotoxic activity causing vacuolation and death of cultured cells[213–215] and may be responsible for the enterotoxic activity of CT-negative strains. Homologous hemolysins with enterotoxin properties have also been identified in other *Vibrio* species.[216–219] However, E1 Tor hemolysin alone is not sufficient to produce illness.

Strains producing this hemolysin that are able to colonize the intestine do not necessarily produce diarrhea.[209] Also, mice given intranasal administration of *V. cholerae* containing deletions within hemolysin genes still experienced acute inflammatory responses.[220] Zonal occludens toxin and accessory cholera toxin genes as well as Shiga-like toxin and various other hemagglutinins and hemolysins (whose contribution to pathogenicity have not been resolved) have also been found in non-O1/O139 strains.[221] The production of a polysaccharide capsule has been found in over 90% of nonepidemic *V. cholerae* strains; heavier encapsulation has a strong association with septicemia.[187] The capsule confers resistance to serum bactericidal activity.[222]

8.5.1.2. V. parahaemolyticus. The most important virulence factor to date is the thermostable direct hemolysin (V_p-TDH), which is responsible for the β-hemolysis seen when these isolates are plated on Wagatsuma agar. Original studies in Japan showed that this phenomenon, named the Kanagawa phenomenon after the prefecture in Japan where it was discovered, was noted in 96% of clinical isolates but only 1% of environmental isolates.[200] It has been found that one-point mutation in the promoter of *tdh* genes in Kanagawa-negative clones can generate a Kanagawa-positive subclone.[353] This result may give insight into disparities in the Kanagawa reaction found in clinical vs. environmental isolates. Volunteer studies also demonstrated the importance of Vp-TDH: Kanagawa-positive strains produced diarrhea, whereas doses of up to 10^9 Kanagawa-negative strains failed to do so in 15 volunteers.[222] Furthermore, studies with isogenic mutants have shown that deletion of the Vp-TDH gene results in loss of enterotoxic activity in laboratory models (using chamber and rabbit ileal loop assays).[223] Kanagawa-negative isolates produce another group of important virulence factors, hemolysins known as V_p-TDH related hemolysins or V_p-TRH. These hemolysins are genetically related to V_p-TDH (they share around 70% sequence homology) but are more diverse (<2.8% divergence among most V_p-TDH vs. 16% between two subgroups of V_p-TRH).[224] Also present is a third hemolysin, thermolabile hemolysin (TLH), also called the lecithin-dependent hemolysin (LDH). The role of LDH in pathogenicity has not been elucidated. Urease production is also linked to virulence. The urease gene cluster lies in close proximity to the V_p-TRH gene on the *V. parahaemolyticus*chromosome, but does not influence the expression of this gene (or V_p-TDH).[225,226] Urease-positive strains that are Kanagawa-negative and lack V_p-TDH and V_p-TRH genes have shown enteropathic activity in a ligated rabbit ileal loop model.[227] Other virulence factors reported over the years include an enterotoxin, a cytotoxic shigatoxin, and various enzymes and adhesins.[228]

V. parahaemolyticus can cause a dysentery-like syndrome, indicating that they may also produce illness by invasion of the intestinal wall. Limited animal studies support this assertion; however, invasion has been demonstrated in vitro and a functional type III secretion system has been characterized.[229–232]

8.5.2. Immunity. Multiple serotypes of *V. parahaemolyticus* can be isolated from a patient's stools, and frequently more than one strain is isolated from an incriminated food.[233] While there has been documented immune response to *V. parahaemolyticus*, it is not known whether infection results in immunity or whether such immunity is serotype- or species-specific.[234] The fortuitous occurrence of two outbreaks of *V. parahaemolyticus* during a single Caribbean cruise demonstrates that it is possible for infection to occur more than once in a given individual within a relatively short period of time (6 days).[202]

8.6. Patterns of Host Response

8.6.1. Nonepidemic *V. cholerae*. Three distinct clinical syndromes—gastroenteritis, wound infections, and primary septicemia—are seen in patients affected by nonepidemic *V. cholerae*.

The severity of gastroenteritis produced by these organisms varies from asymptomatic colonization to heavy, cholera-like purges. In studies of sporadic illness where cases that have been identified retrospectively on the basis of stool cultures, illness has been relatively severe. Among 14 sporadic cases identified in a study in the United States, the median duration of illness was 6.4 days.[196] All of these patients had diarrhea, 71% had fever, but only 21% had nausea and vomiting. One quarter of these patients had bloody diarrhea. In a group of 19 sporadic cases in Bangladesh, the median stool volume was less than 1 l, with a maximum of 8 l; the median duration of illness was less than 24 h, with a maximum of 2.5 days.[235] Experimental infections in normal volunteers and cases identified through outbreak investigations have shown less severe illness, with median durations of 12–24 h and with diarrhea generally lasting less than 4 days.[186]

Septicemia with nonepidemic *V. cholerae* generally occurs in immunocompromised patients, particularly those with liver disease or hematologic malignancies. The case-fatality rate among reported cases has been over 50%. Strains responsible for septicemia have been uniformly found to be heavily encapsulated.

Nonepidemic *V. cholerae* have been isolated from wounds, ears, sputum, urine, and cerebrospinal fluid. In many of these cases, a variety of other potential pathogens

have also been isolated from the same site, making it uncertain that the nonepidemic *V. cholerae* was the true pathogen.[186]

8.6.2. *V. parahaemolyticus.* *V. parahaemolyticus* produces a spectrum of illness very similar to that of nonepidemic *V. cholerae*. Gastroenteritis is the most common syndrome, with diarrhea (98%), abdominal cramps (89%), nausea (76%), and fever (52%).[195] Bloody stools were noted in 29% of *V. parahaemolyticus* gastroenteritis cases between 1973 and 1998,[195] as well as in cases in India and Bangladesh. In both the United States and Japan, illness tends to be mild, with a median duration of 3 days and a very low case-fatality rate. Of the 1,064 *V. parahaemolyticus* illnesses reported in the United States between 1973 and 1998, only one patient died.[195] Outbreaks of severe gastrointestinal illness have been reported. Hypotension and shock occurred in three of five cases in one outbreak in Great Britain,[236] and there were 20 deaths among the 272 patients involved in the Japanese outbreak where *V. parahaemolyticus* was first identified.[237]

V. parahaemolyticus also causes infection in wounds exposed to seawater. Such infections can disseminate and cause sepsis. A surge in wound infections was observed in the aftermath of a severe Gulf Coast hurricane in 2005, Hurricane Katrina, that disrupted sewage systems and displaced thousands of people, some of them wading through contaminated water to get to safety. During the 2 weeks following Hurricane Katrina, 22 new cases of *Vibrio* infections were reported, with 82% of those being wound infections; of the wound infections, 18% were caused by *V. parahaemolyticus* (82% were caused by *V. vulnificus*).[238] Primary septicemia has also been reported, but is not seen as commonly as with *V. vulnificus* or nonepidemic *V. cholerae*.

8.6.3. Other Pathogenic *Vibrio* Species. *V. fluvialis, V. furnissii, V. hollisae*, and *V. mimicus* all cause gastroenteritis. In a Gulf Coast study, *V. fluvialis* was particularly likely to produce bloody diarrhea (six of seven cases).[189]

The principal clinical manifestations of *V. vulnificus* include primary septicemia and wound infections, with a minority (5%) experiencing only gastroenteritis.[167] One-third of patients with primary septicemia present in shock; 70–90% have characteristic bullous skin lesions. The mortality rate for persons with primary septicemia exceeds 50%. Sixty-one percent of the fatal cases report an underlying chronic liver disease.[167] For patients who present in shock, mortality exceeds 90%. Survivors often have multiple organ system failure, requiring protracted hospitalization and rehabilitation. Wound infections are not caused by consumption of raw shellfish. During Hurricane Katrina, 82% of wound infections were caused by *V. vulnificus.*[238] Lesions are typically present on the extremities, most frequently on the legs. Wound infections range from mild, self-limited infections to severe, rapidly progressive myositis and fasciitis.[165]

9. *Aeromonas* Species

9.1. History

Microbiologists have studied aeromonads for over a century. Sanarelli was the first to observe these organisms, which he had isolated from a frog in 1891 and named *Bacillus hydrophilus.*[239] Although early studies showed that direct inoculation produced septicemia and death in animals, the role of these organisms as human pathogens was not recognized until the 1960s. Interest in aeromonads grew considerably with the first publication in 1968 of a review of 28 cases of human *Aeromonas* infections.[233] Although work since then has confirmed the pathogenicity of these organisms, controversy still remains as to the role of aeromonads in gastroenteritis because there have been a lack of volunteer trials and animal models for this disease. However, there now exists a wealth of epidemiologic support for the role of some *Aeromonas* species in foodborne gastroenteritis. *Aeromonas* has become increasingly important in foodborne illness, now being listed on both the FDA "Bad Bug List"[240] and the EPA "Drinking Water Contaminant Candidate List".[241]

9.2. Microbiology

9.2.1. Biological Characteristics of the Organism. Aeromonads are facultatively anaerobic, gram-negative rods with single polar flagella. Species that are pathogenic in humans are oxidase-positive, catalase-positive, and reduce nitrate to nitrite. All ferment D-glucose and most will produce gas in doing so (hence their name). *Aeromonas* species can be divided into two groups: mesophiles, which grow well in a wide variety of temperatures (10–42°C), and psychrophiles, which grow best at lower temperatures (less than 35–37°C). Species in this latter group are not pathogenic in humans but can cause disease in cold-blooded vertebrates and fish.[242]

The taxonomy of the *Aeromonas* genus, which until the mid-1970s was thought to consist of three species, has become complex, with more than 17 phenotypic species now known to exist.[1,242,243] The application of DNA hybridization techniques has led to the division of some phenotypic species (such as *A. sobria*) into more separate genospecies (*A. veronii, A. jandaei*, and *A. schubertii*, in this case).[244] Of *Aeromonas* species, three—*A. hydrophila, A. caviae*, and *A. veronii* biotype sobria—account for more than 85%

of all clinical isolates from humans. Another three—*A. jandaei, A. schubertii*, and *A. veronii*biovar veronii—are considered minor human pathogens. Because of their phenotypic similarities, the genera *Aeromonas* and *Vibrio* were originally placed into a single family (Vibrionaceae) along with one other genus, *Plesiomonas*. However, increasing genetic evidence suggested that *Aeromonas* was so distinct from genera in other families including Vibrionaceae or Enterobacteriaceae that it should constitute its own family.[245] Therefore, in the 2nd edition of *Bergey's Manual of Systematic Bacteriology-Vol. 2*, *Aeromonas* spp. forms the Aeromonadacae family.[1]

The ecology of aeromonads is similar to that of *Vibrio* species. They are autochthonous (free-living) organisms that can be isolated from freshwater, brackish water, occasionally from seawater, and also from the fish, fish eggs, and shellfish that inhabit these environments. However, their isolation from cold-blooded animals, soil, tree bark,[246] ticks,[247] and various other foodstuffs demonstrate that they are not just found in aquatic environments. Their numbers vary seasonally, being highest during the warmer months of May through November. Most foodborne outbreaks are associated with mesophilic species, which can grow at refrigeration temperatures with an optimal growth temperature of 28°C.[248]

9.2.2. Laboratory Diagnosis. *Aeromonas* species are hardy organisms and will grow on a large variety of media, though they may be overlooked on certain semiselective media such as Hektoen enteric or MacConkey agars because some are sucrose-positive (most *Aeromonas* species) or lactose-positive (*A. caviae*). Blood agar has the advantage of allowing easy testing of colonies for oxidase and indole production and also permits the identification of hemolytic species (*A. hydrophila* and *A. veronii*).[249] The addition of ampicillin makes this medium more specific, but excludes *Aeromonas* species that are ampicillin-susceptible (including all *A. trota* and 15–57% *A. caviae* isolates). Modified cefsulodin–irgasan–novobiocin (CIN) agar, which is commonly used for *Y. enterocolitica*, has also been shown to be useful for *Aeromonas.*[250] Both ABA and CIN media are recommended for optimal isolation.[249] A European study looked at *Aeromonas*isolation on xylose–galactosidase medium (XGM).[251] Aeromonas was isolated more frequently on XGM agar (36%) than from any other medium except CIN (43%), but XGM had fewer false positives (11% for XGM vs. 60% for CIN). Enrichment on alkaline peptone water (pH 8.5) (also used for *Vibrio* species) is useful for stools obtained after the acute phase of the illness, when numbers of viable organisms have usually dropped considerably. Gram-negative and Selenite–F broths used for enrichment of *Salmonella* and *Shigella* can also be used for aeromonad recovery.[242] Symptoms of *Aeromonas* sp. infection range from mild gastroenteritis to wound infections. Therefore, the decision to do species or broad complex-level identification (*A. hydrophila*, *A. caviae*, or *A. veronii*) of aeromonads depends on the clinical outcome of the cases from which they were isolated, and because the identification of aeromonads in fecal specimens may not be involved with diarrheal disease (see below), the relative quantity of recovered isolates should be reported as well.[252]

One recurring problem is that *Aeromonas* isolates are frequently mistaken for halophilic vibrios. *Aeromonas* can be differentiated from vibrios using preparations containing 6% NaCl in which *Aeromonas* cannot grow. *Aeromonas* is also resistant to 150 μg vibriostatic agent (O/129).[248] Nevertheless, there are exceptions to these rules. Serotyping and additional testing are recommended to differentiate *A. veronii*biovar veronii from *V. cholerae.*[252] *A. caviae* is particularly difficult to separate from *V. hollisae*; additional salt tolerance and esculin hydrolysis tests are needed.

Phenotypic species identification can be difficult in the case of *Aeromonas*: most commercially available kits are inadequate for this.[248,253] In outbreak investigations or for unusual clinical cases, genospecies identification should be attempted.[254] Several molecular techniques have been used in genotyping. PCR of cytolytic and cytotonic genes is used in some laboratories.[255,256] RFLP and PFGE of housekeeping genes are also used.[257] Serotyping may also be useful in epidemiologic investigations.[258,259]

9.3. Descriptive Epidemiology

Aeromonas species has become increasingly recognized as a common cause of enteric infections. *Aeromonas* species are frequently isolated from stools of patients with diarrhea and, in some studies, have been the most frequently isolated potential pathogen.[239,260] One epidemiologic study found that *Aeromonas* was the only pathogen isolated in diarrheic infants.[261] International studies have indicated the incidence of *Aeromonas* in children at 2.3% in Taiwan 2.8% in Switzerland and as much as 13% in Nigeria and 50% in Peru.[262–265] In an even more recent Iranian case–control study, 3.9% of pediatric diarrheal patients were found to harbor *Aeromonas* spp. as the sole pathogen in stool cultures, but no *Aeromonas* was isolated from controls.[266] Rates tend to be lowest in industrialized countries and highest in developing countries indicating that sanitary conditions may be one determining factor in the epidemiology of this infection. In Sweden, *Aeromonas* was found in 2% of adult diarrheal infections, the most common risk factor being travel.[267] *Aeromonas* has been implicated in traveler's diarrhea in a number of other studies as well.[268–270] In the United States, surveys have found isolation rates of 2.5–7.1% in patients

with diarrhea. At least one study indicates that males may be preferentially affected by *Aeromonas* septicemia.[271]

It is worth noting, however, that Aeromonads can be found in up to 8% of asymptomatic individuals; and excretion rates of 0–2% have been recorded in healthy individuals.[272] Furthermore, although some studies have shown that *Aeromonas* is more common in stools of symptomatic patients, several studies have found *Aeromonas* to be equally common in asymptomatic individuals.[260] Other potential pathogens are often found in stools testing positive for *Aeromonas.*[265,273] As of yet, a reliable animal model for *Aeromonas* has not been identified.

Isolation from stool and from extraintestinal sites (predominantly blood and wounds) is more common in the warmer months of the year when aeromonads are present in higher numbers in the environment and contact with water and consumption of *Aeromonas*-carrying foods may be higher.[239,271] The prevalence of *Aeromonas* species varies by geographic location. *A. caviae* has reportedly been the most frequently isolated species in parts of Bangladesh[274] and the United States and Europe.[275] Other studies have shown that *A. sobria* and *A. hydrophilia* are the predominate clinical and environmental isolates in southern India,[273] Australia, Thailand, the Netherlands, and Bangladesh.[275]

In California, where reporting of *Aeromonas* infections has been mandatory since 1988, a 1992 review showed the annual incidence to be 1.1 per 100,000 in all age groups combined and around 5 per 100,000 for children less than 2 years old.[276] The most common site of isolation was the gastrointestinal tract (85%), followed by wounds (9%). *Aeromonas* is also more commonly found in both elderly and immunocompromised individuals.

9.4. Mechanisms and Routes of Transmission

Aeromonas is usually associated with the consumption of cooked prawns, raw fish, or raw oysters.[277–279] For most of these cases, suspected foods are usually prefrozen or improperly stored and then inadequately heated before consumption.[248] *Aeromonas* has also been detected in milk, vegetables, poultry, and meat. Under experimental atmospheric conditions, *Aeromonas* has been shown to grow rapidly in lettuce[280] and can also grow well in milk.[281]

Studies of sporadic *Aeromonas* infections have revealed different risk factors. One study in the United States found consumption of untreated well water to be associated with this infection;[282] a second study found both untreated well water and prior antibiotic therapy to be risk factors.[283] An outbreak in a children's hospital ward in India found equivalent *A. veronii* biotype isolates in a sink; because the outbreak evolved over the course of a month, person-to-person transmission was suspected.[284] Further studies have found that flies harbor[285] and transmit *Aeromonas* from fly-to-fly and fly-to-food.[286]

9.5. Pathogenesis and Immunity

While *Aeromonas* species produce a wide variety of extracellular virulence factors, two categories of extracellular molecules are believed to play a role in the pathogenesis of *Aeromonas*: colonization factors and enterotoxin production. The type IV pilin, Bfp, is thought to promote colonization as it has been shown to mediate adherence to enterocytes.[287] Adhesion properties of *Aeromonas* strains have been found to correlate with production of other virulence factors.[288] It has been reported that only 24% of *Aeromonas* isolates, especially those obtained from clinical specimens, secrete enterotoxins, which are categorized into two general groups: cytolytic and cytotonic.[242,289,290] Of the cytolytic enterotoxins there is a well-characterized pore-forming aerolysin toxin and cytotoxic enterotoxin (Act). Like aerolysin, Act is hemolytic and pore-forming, but it also induces a number of host inflammatory responses and stress factors. Act is lethal when injected into mice intravenously.[291] Although both aerolysin and Act share similar function, they differ in sequence and structure. Two variants of the toxins are believed to exist. One variant constitutes the aerolysin family of 49–55 kD enterotoxins. The other class of enterotoxin is composed of 64–69 kD proteins containing 45–51% sequence homology to the HlyA hemolysin of *V. cholerae*;[292,293] studies have shown that distribution of this hemolysin gene varies by *Aeromonas* species.[294] Thus far, cytotonic enterotoxins have been shown to have different molecular weights and physiologic properties.[291] Both a heat-labile (Alt) and a heat-stabile (Ast) enterotoxin have been well characterized by the Chopra laboratory.[272] They have been shown to induce fluid secretion in rabbit ileal loop assays and increase cAMP and PGE_2 levels in the intestine. Past serological, cell, and animal assay evidence suggested that cytotonic enterotoxin production may occur in only a small fraction (<6%) of environmental isolates,[295,296] but since then new reports using DNA techniques have shown that 75% of food-poisoning outbreak strains in Norway contain the *alt* gene.[256] Pathogenic mechanisms of other types of *Aeromonas* infections are induced by a variety of other hemolysins, adhesins, proteases, and lipases. Type III secretion systems, which inject virulence proteins directly into host cells, and quorum sensing mechanisms, which regulate virulence factor production based on the density of bacteria, may also be important for the colonization and invasion of host cells.[272] Type III secretion systems have

been shown to play a greater role than Act in host cell cytotoxicity during early (2 h) bacterial–host cell interactions, but Act contributes more during the later stages of infection.[297] The hemolytic uremic syndrome caused by *Aeromonas* is likely due to a Shiga toxin, but more studies are required for confirmation of its existence. In general, it has been found that pathogenic strains isolated from diarrheal patients express toxins better than those found in asymptomatic controls.[277,288]

9.6. Patterns of Host Response

There appears to be a correlation between species type and clinical outcome. Species most commonly associated with gastroenterititis are *A. veronii* biovar sobria, *A. hydrophila*, and *A. caviae* and to a lesser extent *A. veronii* biovar veronii, *A. trota*, and *A. jandaei*.[248] Gastroenteritis varies considerably in severity and is usually self-limited.[261,282,298–301] Watery diarrhea, with a median of eight bowel movements per day, is often associated with abdominal pain (60–70%), vomiting (20–40%), fever (20–40%), and nausea (20%).[257] The diarrhea is occasionally so severe that it resembles cholera, with up to 40 stools per day. A minority of patients with *Aeromonas* gastroenteritis will develop a dysenteric syndrome resembling shigellosis, with blood and mucus in the stools. Children with this form of illness often require hospitalization. There have also been descriptions of a third, much less common syndrome of subacute or chronic diarrhea lasting for more than 10 days and up to several years.[302] Work in Bangladesh has shown that loose stools are most often associated with *alt* gene-containing isolates and more severe manifestations were caused by strains that harbored both *alt* and *ast* genes.[303] Reports of successful antibiotic treatment of patients with this syndrome lend support to the notion that *Aeromonas* is a true gastrointestinal pathogen.

Wound infections, the second most common clinical manifestation due to *Aeromonas*, usually (but not invariably) occur after exposure of open wounds to environmental water sources[304–306] and are most frequently associated with *A. schubertii* and *A. veronii* biovar sobria (in cases of medicinal leech use).[248] Cellulitis is the most common pattern but more severe infections can occur in immunocompetent individuals, including myonecrosis with gas formation that is rapidly fatal without aggressive treatment. These types of infections were seen among survivors of the 2004 Asian tsunami.[307] Treatment must be considered carefully, as *Aeromonas* infections tend to mimic staphylococcal or streptococcal soft-tissue infections due to high pathogenicity and resistance to penicillin. *Aeromonas* are not susceptible to empirical antibiotic treatment commonly used in wound infections, however, it has been successfully treated with fluoroquinolones, third-generation cephalosporins, and cotrimoxazoles.[306]

In immunocompromised individuals, especially those with malignancy, hepatobiliary disease, and diabetes, bacteremia has been described as well as other forms of invasive disease such as bacterial peritonitis. Most of those with *Aeromonas*-bacteremia have *A. hydrophila*, *A. veronii* biovar sobria, and *A. jandaei* infections. However, a case of septicemia was reportedly caused by *A. veronii* biovar veronii strain.[308] Other clinical syndromes reported in the literature include osteomyelitis, ocular infections, pelvic infections, and pneumonia.[239,309–311] Hemolytic uremic syndrome and renal complications have been reported as sequelae of *Aeromonas* gastroenteritis,[312–315] as well as relapsing intermittent diarrhea.[252]

10. *Plesiomonas*

10.1. History

Plesiomonas was first described by Ferguson and Henderson[316] in 1947, who noted that the properties of this organism resembled those of *Shigella*. Originally named simply "C27," this species was later placed variously in the genera *Pseudomonas, Fergusonia, Scatamonas, Vibrio*, and *Aeromonas*, until 1962, when it was definitely placed into a genus by itself, *Plesiomonas*.[317] The name is derived from the Greek word for "neighbor" and was chosen because of the close relationship that was thought to exist between this genus and the genus *Aeromonas*. Although the genus *Plesiomonas* originally resided in the Vibrionaceae family, recent phylogenetic data revealing that plesiomonads are more closely related to Enterobacteriaceae resulted in their transfer to this family.

10.2. Microbiology

10.2.1. Biological Characteristics of the Organism. *Plesiomonas*, unlike *Aeromonas*, has just one hybridization group, *P. shigelloides*. Like *Aeromonas*, *P. shigelloides* are also facultative anaerobic, gram-negative rods that are positive for oxidase, catalase, and nitrite reduction. They are highly motile by means of two to five polar flagella. Although they ferment D-glucose, they do not produce gas in doing so. The species name, *shigelloides*, is due to its antigenic similarity to *Shigella* species, especially *S. sonnei*.[318,319] *P. shigelloides* is susceptible to O/129, a vibriostatic agent used to distinguish *Vibrio* spp. from *Aeromonas* spp. and other gram-negative oxidase-positive bacilli. *P. shigelloides* is unable to grow on 6% NaCl.

P. shigelloides is primarily a freshwater organism, though it can also be found in estuaries and can occasionally be isolated from seawater. It can also be recovered from sediments, fish, shellfish, and occasionally in the feces of asymptomatic cold-blooded and warm-blooded animals. Isolation of *P. shigelloides* is highest during warmer months.

10.2.2. Laboratory Diagnosis. Because this species, unlike most enteric organisms, can use inositol as a carbon source,[320] agar containing inositol–brilliant green–bile salts is particularly useful. Isolation media may also contain xylose-sodium deoxycholate-citrate. Selective enrichment in alkaline peptone water is also useful for *Plesiomonas* in certain situations. Despite the large number of similarities between *Plesiomonas* and *Aeromonas*, a barrage of tests can be used to differentiate the two, including exoenzyme production, myoinositol fermentation, and decarboxylation of ornithine.[248] Commercial preparations are also available for rapid detection.[321] Because *P. shigelloides* grows optimally at 38–39°C, low temperatures (42–44°C) can be used for its isolation in samples suspected to contain aeromonads. An international serotyping system is available for epidemiological investigations,[322] which employs over 100 serovars using combinations from 76 O and 41 H antigens.[248]

10.3. Descriptive Epidemiology

There is considerable epidemiological evidence supporting the role of *Plesiomonas* as an enteropathogen.[323–325] Two outbreaks have been identified in which predominant serotypes (O17:H2 in one, O24:H5 in another) have been identified,[323] and an outbreak of gastroenteritis caused by *P. shigelloides* and *Salmonella* was reported by the CDC, in which contaminated water and potato salad were thought to be the vehicles.[324] Plesiomonas has also been reportedly isolated from Chinese soldiers.[326] In further support of the enteropathogenic role of *Plesiomonas*, when asymptomatic controls have been studied, carriage rates have usually been less than 1% (although higher in developing countries). In most reported cases of gastrointestinal disease, *Plesiomonas* has been the predominant organism in the stool. Other pathogens are generally not found when sought. Several case reports have noted clinical improvement and disappearance of *Plesiomonas* from stool cultures after treatment with antibiotics to which *Plesiomonas* is susceptible.[327,328] Virulence of *P. shigelloides* has not been confirmed by experimental studies.

The incidence of *Plesiomonas*-associated diarrhea is largely unknown. In a study of stool specimens submitted to a Canadian reference laboratory, the isolation rate was similar to that of *Aeromonas* (25 out of 17,820 specimens).[324,328] A study in Taiwan reported that one-third of pediatric patients harboring *Plesiomonas* also had other enteric pathogens isolated from stool specimens.[329] Thirty-five percent of the cases occurred in children 2 years of age or younger.[329] Despite this, *Plesiomonas* can affect all age groups. *Plesiomonas* affects all health conditions as well. However, rates of *Plesiomonas* infections in HIV-positive populations of Taiwan have shown an increase;[330] also extraintestinal infections associated with immunocompromised health status (esp. underlying biliary tract or liver disease) are becoming more apparent.[331]

10.4. Mechanisms and Routes of Transmission

Several possible *Plesiomonas*-related outbreaks have been reported, mostly from Japan. The most common vehicles implicated are raw or undercooked fish or shellfish. Chicken and water have also been implicated.

Sporadic *Plesiomonas* gastroenteritis has been linked to both seafood consumption and foreign travel.[324,327,328] In a case–control study of sporadic illness, published by the CDC, both of these factors correlated with infection ($p<0.001$ for both); 21 of the 31 cases had one of both of these risk factors.[327] A 1997 Japanese study revealed that *P. shigelloides* is the third most common cause of traveler's diarrhea in that country.[268]

Like *Aeromonas*, *P. shigelloides* is unlikely to grow in foods with high acidity or salt content (more than 3–3.5% wt/wt).[248]

10.5. Pathogenesis and Immunity

Little is known about virulence factors in *Plesiomonas*. No colonization factors such as pili have yet been described. A β-hemolysin has been found and is reported to be present in more than 90% of isolates.[332] This hemolysin may have enterotoxic activity similar to that of aerolysin and may be important in making iron available to the bacteria. Culture filtrates from aquatic isolates were shown to cause intracellular vacuolation on mammalian cells and produce hemolytic and enterotoxic activities.[333] Also, a complex of three heat-stabile LPS-binding proteins and LPS was column purified from *Plesiomonas* culture filtrates. The protein portion had cytotoxic and enteropathogenic activity.[334] Adhesion and invasion by *P. shigelloides* have been reported.[335] Factors involved in adherence and invasion are unknown.

10.6. Patterns of Host Response

Three general clinical manifestations of enteric *Plesiomonas* infection have been described: (1) Watery diarrhea is the most common form, with abdominal pain and some-

times with vomiting or fever. (2) Some experience a more prolonged subacute diarrhea lasting anywhere from 14 days to 3 months. (3) Frank mucus or blood has also been detected in an invasive form of *P. shigelloides* more similar to colitis.[321,336] Most patients experience severe abdominal pain and cramping; and 25–40% of patients experience fever and/or vomiting.[321] Children in Bangladesh have exhibited watery diarrhea (84%), dysentery (16%), vomiting (71%), and fever (8%), with duration being 2 weeks or longer.[337]

Many forms of extraintestinal infection have been reported. Meningitis is the most common of these and is seen in neonates.[338] Septicemia and bacterial peritonitis have been reported in adults, especially those with cirrhosis or who are otherwise immunocompromised.[338] Septic arthritis, endophthalmitis, and cellulitis have been described.[336]

11. Control and Prevention

11.1. General Concepts

11.1.1. Food Safety Regulation in the United States. USDA and FDA are the primary government organizations charged with protecting the food supply. Until recently, regulation of the safety of meat and poultry products was done largely through an inspection system (begun in 1906) that relied heavily on the visual examination of carcasses. This procedure is still done, but to update the inspection system, in 1995 the USDA required meat and poultry processing plants to adopt a system known as HACCP (hazard analysis and critical control points). HACCP is endorsed by the FDA, which regulates the majority of other food products not regulated by, USDA. HACCP is also endorsed by the National Academy of Sciences, the Codex Alimentarius Commission (an international food safety organization), and the National Advisory Committee on Microbiological Criteria for Foods. Regulations were put in place in December 1995 by the FDA requiring the use of HACCP in certain seafood processing operations. FDA also established HACCP regulations for the juice industry in 2001. Pilot HACCP studies involving cheese, bread, flour, and other products are currently in progress.

The HACCP system provides a rational approach to the control of microbial hazards in food. First presented at the 1971 National Conference on Food Protection, the HACCP system consists of seven principles: (1) identification and assessment of hazards associated with growing, harvesting, processing, marketing, preparation, and use of a given raw material or food product; (2) determination of critical control points to control any identifiable hazard; (3) establishment of preventive measures with limits for each control point; (4) establishment of systems to monitor critical control points; (5) establishment of corrective actions; (6) verification of properly working systems; and (7) effective recordkeeping.[339] When used as part of a regulatory system, HACCP is generally combined with microbial or other standards, to provide verification that the production process is "under control."

Some food products, like eggs, are covered by multiple jurisdictions. State, county, and local health departments have jurisdiction over food-preparation facilities, such as restaurants, cafeterias, and other places where food is prepared for public consumption.

Fresh produce has become increasingly implicated in foodborne outbreaks. Appropriate approaches to development of regulatory systems for produce are under investigation. Currently, FDA relies primarily on Good Agricultural Practice guidelines for minimizing risk associated with such products. However, the recent proliferation of outbreaks has raised questions about the effectiveness of this approach, and the need for develop of HAACP systems for produce.

11.1.2. Methods to Further Reduce Foodborne Bacterial Disease

11.1.2.1. Control of Pathogens in Animal Reservoirs. For zoonotic pathogens such as *Salmonella, Campylobacter*, and STEC that colonize the gastrointestinal tracts of animals without causing illness, elimination may be difficult. Crowded feeding lots and animal housing facilities promote cyclic reinfection and continuation of these pathogens. Furthermore, prolonged use of antibiotics may promote the growth of resistant pathogens. However, it may be possible to reduce the prevalence of these organisms on farms and in individual animals. As more is learned about the ecology of these organisms, better control measures can be instituted. For example, epidemiological studies have shown that *Campylobacter* may be introduced into chicken houses through contaminated water. Therefore, use of chlorinated water may decrease the prevalence of *Campylobacter*. Vaccination of chickens against *Campylobacter* may be another useful intervention. Another example of this sort of protective measure is the 2003 California ban of oysters imported from the Gulf Coast during certain months unless they are treated to reduce harmful bacteria, such as *Vibrio* spp. Treatment involves two FDA-approved methods: low-temperature pasteurization and high-pressure processing.[340]

Other foodborne pathogens have been successfully controlled or eliminated in animal reservoirs. A brucellosis eradication program by the USDA eliminated *Brucella melitensis* from sheep and has markedly decreased the prevalence of *B. abortis* in cattle. Other opportunities to con-

Table 8. Prevalence of Various Pathogens Sampled at Slaughter Plants in the United States

Pathogen	Product sampled			
	Steer/heifer carcasses[a] (2089 samples) (%)	Raw ground beef[b] (563 samples) (%)	Raw ground chicken[c] (285 samples) (%)	Raw ground turkey[d] (296 samples) (%)
C. perfringens	2.6	53.3	50.6	28.1
S. aureus	4.2	30.0	90.0	57.3
L. monocytogenes	4.1	11.7	41.1	30.5
C. jejuni/C. coli	4.0	0.002	59.8	25.4
E. coli O157:H7	0.2	0	0	0
Salmonella	1.0	7.5	44.6	49.9

[a]Nationwide Beef Microbiological Baseline Data Collection Program: Steers and Heifers 1994.(347)
[b] Nationwide Federal Plant Raw Ground Beef Microbiological Survey 1996.(348)
[c] Nationwide Raw Ground Chicken Microbiological Survey 1996.(349)
[d] Nationwide Raw Ground Turkey Microbiological Survey 1996.(350)

trol pathogens in animals include controlling breeding stock, using effective vaccines, and implementing other on-farm controls to decrease carriage of pathogens.

11.1.2.2. Control of Pathogens in Plants. The control of pathogens in plants is complicated by the persistence and survival of some organisms in the environment. Irrigation and flood water or manure containing untreated animal or human waste can be potential sources of contamination. Prevention of bacterial pathogens in certain produce is difficult due to large surface areas and certain plant structures that can accumulate bacteria. Once internalized, the pathogens are difficult to remove. Scientists are investigating new technologies that would eliminate contaminants from fresh produce. Some suggestions have included irradiation of high-risk foods and washing produce with chemically altered water.

11.1.2.3. Control of Pathogens During Food Production. A number of innovative approaches are currently being used to prevent or minimize contamination of meat, poultry, and seafood by pathogens. The major source of pathogens on meat and poultry is the gastrointestinal tract of animals (with subsequent transfer to the hide or feathers), although environmental organisms can also be found on these foods. Table 8 shows results of a microbiological survey of raw meat products conducted by the USDA. For fish and other seafood, which frequently must be harvested under difficult conditions and at varying distances from processing, transport, and retail facilities, contamination may be from numerous sources, including the environment. In animal slaughter establishments, several methods are being used to reduce microbial contamination on carcass surfaces, including washing exterior surfaces of carcasses prior to slaughter, steam vacuuming of carcass surfaces during slaughter, and applying antimicrobial treatments to carcass surfaces immediately after slaughter.

11.1.2.4. Improvement of Food-Handling Practices. Food-handling errors frequently contribute to foodborne illness, allowing pathogens already present on foods to multiply to levels sufficient to cause illness. Such errors identified in outbreak investigations during a 15-year period included improper holding temperature (87%), poor personal hygiene (59%), inadequate cooking (56%), and contaminated equipment (47%).(8)

Pathogens on raw food products are the major source of foodborne illness. Independent retail food studies have shown varying levels of pathogen prevalence in raw produce and raw poultry and meats. Persons handling and preparing raw food products, including the producer, processors, food service personnel, and the public, must have the knowledge necessary to control microbial hazards. The level of knowledge needed by each group depends on the role it plays in the overall process. In homes and food service establishments especially, it must be emphasized that improper food-handling practices, such as leaving foods in ovens at low temperatures or at room temperatures or placing food in large containers prior to chilling, result in foodborne illness.(341)

Targeting specific education programs for persons at high risk should be a part of any consumer education program. For example, pregnant women should be advised of the potential risk of acquiring listeriosis from consuming certain soft cheeses and ready-to-eat foods. Persons with liver disease should be advised to avoid eating raw seafood. Immunocompromised persons, such as those undergoing cancer chemotherapy or those who have AIDS should be advised to avoid specific foods and take extra precautions in food handling and preparation. Consumers are also encouraged to thoroughly wash all raw produce before consumption.

Numerous publications are available from the FDA, FSIS, and other federal, state, and local government agencies on proper food handling. Educators, trainers, and organizations developing education and training materials for food workers and consumers may obtain information from the Foodborne Illness Education Information Center or online at www.foodsafety.gov.

11.2. Antibiotics and Chemotherapeutics

Antibiotics generally have no role in prevention of foodborne illness and in some cases may predispose individuals to infection with gastrointestinal pathogens or post-infection chronic sequelae. Occasionally, treatment of persons infected with such pathogens as *Shigella* may be warranted in order to prevent secondary transmission. Much attention has also been given to the use of antibiotics in animal feed. Subtherapeutic levels of antibiotics are routinely given to livestock in order to reduce animal disease and promote growth. There is increasing scientific evidence for cross-resistance, whereby bacteria in livestock develop antibiotic resistance and humans become infected with the resistant bacteria after consuming or handling contaminated meat. There are programs in place to describe the extent to which antibiotic resistance occurs. One such program, the National Antimicrobial Resistance Monitoring System (NARMS), is a multi-agency program headed by the FDA. NARMS monitors isolates collected from humans, farm animals, and retail meats for changes in antimicrobial drug susceptibilities of select enteropathogens.

11.3. Immunizations

Immunizations, with few exceptions, have little role in the control of foodborne illness. Typhoid fever, which may be spread through the foodborne route, is readily preventable through vaccination (see Chapter 43). New oral cholera vaccines show promise, but are not widely available (see Chapter 12). Vaccination against β-toxin has proved successful in controlling pig-bel in Papua New Guinea.

12. Unresolved Problems

The largest unresolved problem in this group of foodborne illnesses is determining the pathogens that make up the significant number of foodborne disease outbreaks with "unknown" etiology. While some of these outbreaks may be due to known pathogens, others await recognition. Also, the emergence of new pathogenic strains will continue to challenge the medical and public health communities; *E. coli* O157:H7 is an excellent example of this.

Underreporting of disease is another serious public health problem. Because most persons regard diarrhea as an inconvenience rather than a symptom of disease, the vast majority of diarrheal episodes do not result in visits to physicians, even though persons may be incapacitated for several days. A CDC study on self-reported diarrheal illness in FoodNet sites between 1998 and 1999 found that only 28% of individuals with symptoms sought medical care.[342] Lack of stool specimen submission is also a concern. A 1996 physician survey concluded that 44% of physicians requested bacterial stool culture. Other factors that contribute to underreporting of foodborne illness are occasional (rather than routine) laboratory testing for various pathogens and neglecting to report confirmed cases even when required. For these reasons, assessments of the impact of foodborne illness have always relied heavily on estimates and assumptions. A 1999 CDC study used underreporting multipliers to estimate that foodborne disease causes approximately 76 million illnesses and 5,000 deaths each year in the United States.[10] The Economic Research Service (ERS) of the USDA estimates that in 2000 the five major foodborne pathogens (*E. coli* O157, *E. coli* non-O157 STEC, *Listeria*, *Salmonella*, and *Campylobacter*) cost 6.9 billion dollars in medical costs, lost productivity, and premature deaths.[343] Other estimates yield a cost of 17 billion dollars for all foodborne deaths alone.[344]

As the number of goods imported by the United States increase, so does the concern over the safety of these imports. As globalization of the food supply expands, federal agencies must continue to ensure the safety of the food supply by increasing the number of inspections for microbiological and chemical hazards and by improving regulations for production of imported foods.

Finally, consumers and industry must be educated on the adverse effects of improper food handling and ways to eliminate bacteria that may already be present in raw foods. We must also look to new approaches for maintaining sustainable levels of livestock production without the use of veterinary antibiotics that have been linked to human antibiotic resistance.

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CHAPTER 7

Botulism

Patricia A. Yu, Susan E. Maslanka, Michael E. St. Louis, and David L. Swerdlow

1. Introduction

Botulism is a neuroparalytic illness resulting from the action of a potent toxin produced by the organism *Clostridium botulinum*. Foodborne botulism is rare, but it may kill rapidly, and contaminated products may theoretically expose many persons to the toxin. Foodborne botulism therefore represents a medical and a public health emergency that places a premium on rapid, effective communication between clinicians and public health officials.

Depending on the mode of acquisition of the toxin, there are four distinct forms of botulism. Foodborne botulism results from the ingestion of food containing preformed toxin. Wound botulism is caused by organisms that multiply and produce toxin in a contaminated wound. Infant botulism is due to the endogenous production of toxin by germinating spores of *C. botulinum* in the intestine of the infant. Adult botulism from intestinal colonization is difficult to diagnose, but is suggested by those cases in which no food vehicle can be identified, there is no evidence of wound botulism, and there is evidence of prolonged excretion of toxin and *C. botulinum* in stool. Two other forms of botulism include iatrogenic, which occurs less commonly, and bioterrorism associate botulism, which theoretically could occur botulinum toxin was ever used as a bioterror weapon.

The seven botulinum toxin types of *C. botulinum* (A–G) are antigenically distinguishable. Types A, B, E, and, in rare cases, F cause disease in humans. Types C and D cause disease in birds and mammals. Type G, identified in 1970, has not yet been confirmed as a cause of illness in humans or animals. Important epidemiological features and some clinical characteristics distinguish the types of botulism that cause human illness. Rare cases of infant and adult botulism cases have been confirmed to be the result of intestinal colonization by non-botulinum *Clostridium* species that were able to produce botulinum neurotoxin such as *C. baratii* and *C. butyricum*.

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2. Historical Background

In the late 18th century, a syndrome characterized by muscle weakness and respiratory failure linked to eating blood sausage was described and termed "sausage poisoning." Similar outbreaks related to sausage, other meats, and fish were reported in Germany, Scandinavia, and Russia. In time, the syndrome became known as "botulism" after "botulus," the Latin word for sausage.

In 1897, Van Ermengem detected the potent neurotoxin in a ham implicated in an outbreak of botulism in Belgium and isolated an organism that produced the toxin and grew only in the absence of oxygen. In a series of pioneering microbiological experiments, he determined that (1) foodborne botulism is not an infection, but rather an intoxication caused by a toxin produced in the food by the bacterium; (2) the bacterium will not produce toxin in food if the salt concentration is high enough; (3) the toxin is heat-labile; (4) the toxin when ingested with contaminated food is resistant to acid and proteolytic enzymes; and (5) not all animal species are affected equally by ingested toxin.

In 1904, Landman first identified a nonmeat product (home-canned beans) as the cause of an outbreak of botulism. During and shortly after World War I, the practice of home-canning vegetables increased markedly, as did the

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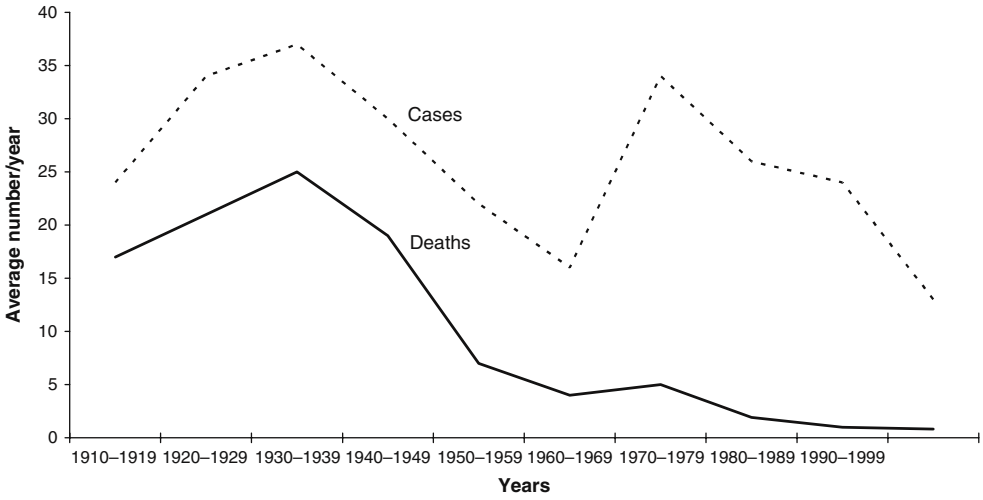


Figure 1. Average annual number of cases and deaths from foodborne botulism, United States, 1899–2006.

number of cases of botulism. Meyer made major contributions to the understanding of botulism during these years, studying the distribution of spores in soil in the United States and Europe, characterizing the epidemiology of botulism transmitted by home-canned vegetables and fruits, and even identifying a new type of *C. botulinum*.[1] In the mid-1940s, with the increased availability of refrigeration and the widespread application of improved preservation methods both in the home and by industry, the incidence of botulism began to decline. In recent years, increases in the incidence of botulism coincided with occurrences of large restaurant-associated outbreaks including 59 cases in 1977 (peppers), 20 cases in 1985 (sautéed onions), 36 cases in 1985 (chopped garlic in soybean oil), and 17 cases in 1994 (skordalia dip) (Figure 1).

Although it had been noted earlier that the toxins of the Van Ermengem and the Landman strains were serologically distinct, it was in 1919 that the first two type designations (A and B) were established. The types were based on strains isolated in the United States and could not be directly related to the earlier European strains, since the latter were no longer available for study. *C. botulinum* types C and D were discovered in relation to outbreaks of botulism in domestic birds and animals. *C. botulinum* type E was first isolated in 1936, from fish implicated in a botulism outbreak. *C. botulinum* type F was first isolated and recognized as a cause of human botulism in 1960. In 1970, *C. argentinense*, formerly *C. botulinum* type G, was isolated from soil in Argentina, but although there have been reports of isolation of the organism from human autopsy specimens, it has not yet been confirmed as a cause of botulism.[2] Neurotoxigenic strains of *C. baratii*[3] and *C. butyricum*[4] have been implicated in type F and type E human botulism, respectively.

3. Methodology

3.1. Sources of Mortality and Morbidity Data

The Centers for Disease Control and Prevention (CDC) maintains surveillance of botulism in the United States. The sources of morbidity and mortality surveillance data include (1) state, territorial, and local health departments (botulism is a reportable disease in all states); (2) state and territorial public health laboratories that contact CDC for laboratory support; (3) the US Food and Drug Administration, which becomes involved if the implicated food item is commercially produced or distributed; (4) the US Department of Agriculture, which becomes involved if the contaminated food item is derived from meat, poultry, or eggs; (5) clinicians who contact CDC for consultation, laboratory support, or antitoxin; and (6) the manufacturers of antitoxin, who are sometimes contacted initially by a patient's physician.

CDC provides epidemiological consultation to state health departments for suspected non-infant botulism cases and laboratory diagnostic services for infant and non-infant cases for samples from state health departments that do not conduct botulism laboratory testing. In addition, CDC monitors and authorizes the release of botulinum antitoxin for non-infant cases. The California Department of Health Services provides clinical consultation, laboratory diagnostic services for infants residing in California, and human botulism immune globulin intravenous (BabyBIG®) to state

health departments for suspected infant botulism cases. In spite of this surveillance, it is possible that underreporting of botulism occurs as a result of misdiagnosis of cases (as, for example, myasthenia gravis, Guillain–Barré syndrome, or stroke). Mild cases of botulism may escape diagnosis because of a lack of overt neurological findings, and even severe cases resulting in death may be diagnosed only after death, if at all.[5,6] Missed cases of botulism are an important public health problem, because lack of recognition precludes epidemiological investigation and prevention of subsequent cases due to the same contaminated foods.[5,7]

3.2. Laboratory Confirmation

Since botulism results from the action of a toxin, the most definitive laboratory confirmation of the disease is the demonstration of the toxin itself in clinical specimens (serum, feces, wound, or gastric aspirate) or foods.[8] Isolation of *C. botulinum* organisms from foods are not definitive, as the organisms may be present in food products that do not support toxin production. On the other hand, isolation of *C. botulinum* from human cultures of feces is very rare except in persons suspected of having botulism, and may be considered strong evidence supporting the diagnosis.[9] Because of the delays inherent in laboratory confirmation, the initial treatment decisions should be made on the basis of patient signs and symptoms that are consistent with botulism.

The mouse bioassay is currently the most sensitive, specific, and commonly used method for detecting botulinum toxin in foods and cultures, and there is no other method approved for testing clinical specimens collected from suspect botulism patients.[8,10] A standard volume of the test specimen (e.g., an aliquot of serum or extract of feces or food) is injected into the peritoneum of test mice and from this site is absorbed into the bloodstream. The same volume of test specimen is combined with selected botulinum antitoxins and injected into the peritoneum of control mice. Experienced laboratory workers can recognize the clinical manifestations of botulism in the mouse. The clinical diagnosis is confirmed if samples injected into mice produce paralytic signs consistent with botulism and mice injected with sample mixed with toxin-specific antitoxin have no paralytic signs. While test results are usually available within 24 h of specimen receipt, very low levels of toxin may not produce botulism signs in mice for up to 96 h. Some medications administered to patients and on rare occasion some food matrices may prevent the observation of botulism signs; however, experienced laboratory personnel can readily differentiate between these non-specific signs and those caused by botulinum toxin. This test can only be performed in select laboratories because it requires animals and experienced laboratory personnel who can perform these tests safely and have the expertise to recognize botulism signs in mice.

Much effort has been made for the development of satisfactory in vitro assays for botulinum toxin. The status of studies on immunodiffusion, passive hemagglutination, immunofluorescence, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), and biosensors have been reviewed.[11] ELISA is more rapid than the mouse bioassay and the technology is readily available in laboratories, however, it is less sensitive than the mouse bioassay, some cross-reactivity may be observed, it only detects four toxin types, and is not currently approved in the United States for clinical specimens.[12] ELISA is an effective, rapid screening method with results confirmed by mouse bioassay. Real-time PCR is a method of rapid detection of all seven botulinum neurotoxin genes in cultures of clinical specimens and foods.[13] However, this test must be used in conjunction with a method that can detect toxin because (1) multiple neurotoxin genes may be detected but the strain may only produce one botulinum toxin type and (2) *C. botulinum* spores are ubiquitous in the environment, including foods, and may be present without being the cause of disease. Mass spectrometry is another in vitro method that is more rapid than the mouse bioassay and is sensitive in detecting and differentiating four toxin types A, B, E, and F.[14] Pulsed-field gel electrophoresis (PFGE) has been used to differentiate strains of *C. botulinum* for molecular epidemiologic analysis.[15,16] Nevertheless, because of the extraordinary biological potency of botulinum toxins, the mouse bioassay due to its superior sensitivity and specificity remains the method of choice for laboratory investigations of botulism. The mouse bioassay is also remarkable in that it allows the detection of any type of botulinum toxin, including any types waiting to be discovered in the future. This is because experienced laboratory personnel can identify the signs of botulism in mice and this identification is independent of the use of antitoxins used for toxin neutralization. Since all in vitro methods developed so far depend on specific antibody to capture toxin (either on microplate or microbeads), the mouse bioassay is the only method capable of demonstrating the action of a new type of botulinum toxin that is antigenically distinct from those currently recognized.

Efforts have been made to prepare laboratories to respond to botulism-related bioterrorism. The Laboratory Response Network (LRN) is a network of state and local public health, federal, and military laboratories in the United States and internationally. The mission of LRN is to maintain an integrated national and international network of laboratories that are fully equipped to respond quickly to acts of chemical or biological terrorism, emerging infectious diseases, and other public health threats and emergencies.[17]

LRN's botulism-related activities include providing to member laboratories agent-specific procedures and standardized reagents and controls, a laboratory referral directory, and secure access for e-mail regarding training and emergency communications.

4. Biological Characteristics of the Organism

C. botulinum is not a single species but rather a group of culturally distinct organisms that are alike only in that they are *Clostridia* and produce antigenically distinct neurotoxins with a similar pharmacological action. The seven types of *C. botulinum* (A–G) are distinguished by immunologically distinct types of toxin. *C. botulinum* organisms have also been divided into group I (proteolytic; type A and some strains of types B and F), group II (nonproteolytic; type E and some strains of types B and F), group III (types C and D, which are closely related to *C. novyi*), and group IV (*C. argentinense*, formerly *C. botulinum* type G[18,19]). *C. botulinum* organisms are straight to slightly curved, gram-positive (in young cultures), motile, anaerobic rods, 0.5–2.0 μm in width by 1.6–22.0 μm in length, with oval, subterminal spores.[20] Demonstration of the ability to produce botulinum toxin is critical to the identification of neurotoxin producing *Clostridia* species since nontoxigenic equivalents exist. *C. sporogenes* are indistinguishable from *C. botulinum* group I strains and show DNA homology, but do not produce toxin. Cases of human botulism due to other species of *Clostridia*, *C. baratii*[3] and *C. butyricum*,[4,21] have further strained the taxonomic classification of botulism-causing organisms. All typical *C. baratii* and *C. butyricum* strains are biochemically identical to the neurotoxin producing strains, but do not contain the genes necessary to produce botulinum toxin.

The specific cultural and biochemical characteristics of *C. botulinum* are described elsewhere.[20,22] Because of the practical importance of temperature, salt concentration (water activity), and pH in the preservation of foods, the effects of these factors on the growth of *C. botulinum* have been thoroughly investigated. All strains of *C. botulinum* are mesophilic, growing best at 40°C; although some nonproteolytic strains have been noted to grow at temperatures as low as 3°C.[23] The organism will generally not grow in an acid environment (pH <4.6) or in foods with a high salt content (water activity <0.93). Temperature, pH, and salt content are interrelated factors; lowering the pH or raising the salt concentration increases the minimum temperature at which spores will germinate or vegetative cells will begin to grow.[24]

The ability of *C. botulinum* to cause food poisoning in humans is directly related to the production of heat-resistant spores that survive preservation methods that kill nonsporulating organisms.[25] The heat resistance of spores varies from type to type and even from strain to strain within each type; although some strains will not survive at 80°C, spores of many strains require temperatures above boiling to ensure destruction.[26,27] The thermal resistance of spores also increases with higher pH and lower salt content of the medium in which the spores are suspended.[28] In general, type A and proteolytic type B spores are more heat-resistant than type E and nonproteolytic type B spores. Spores are also susceptible to some types of radiation.[27,29,30] Nitrites are effective as well in preventing growth from spores that germinate[30] and are used commercially in semiprocessed meats.

C. botulinum types C and D have bacteriophages that are associated with toxigenicity. Nonlysogenic, nontoxigenic mutants have been obtained by "curing" them of their phages. These mutants can be made lysogenic and toxigenic again by infection with phages obtained from other lysogenic strains.[31] Hence, the ability of *C. botulinum* types C and D to produce toxin seems to depend on the presence of specific bacteriophages. No association between bacteriophages and toxigenicity has been found, however, for type A, B, E, and F strains of *C. botulinum*. The association between lysogenization by phage and toxin production has been noted with *Corynebacterium diphtheriae*. The genetic information for the production of neurotoxin by *Clostridium tetani*, however, appears to be located on a plasmid.[32] Recent evidence has found that neurotoxin genes of at least some strains of *C. botulinum* serotypes A and B are located on plasmids.[33]

Toxin is released from *C. botulinum* organisms only after lysis of the cells. The lytic enzymes responsible for this autolysis are located in the cell wall. More than one such bacteriolysin has been identified, and at least some appear to be group specific.[34]

5. Descriptive Epidemiology

5.1. Foodborne Botulism

Botulism has been reported from all parts of the world, although the causative strains of *C. botulinum* and the characteristic food vehicles responsible for botulism may vary widely among regions. Botulism in continental Europe is almost exclusively type B disease and is predominantly caused by eating home-cured hams. The resulting illness is relatively mild and slowly progressive compared with botulism in the United States.[35] Most outbreaks in Japan are type E botulism associated with the consumption of preserved seafood.[36,37] In Canada, type E botulism outbreaks associated with seafood preserved by First Nations and Inuit populations is most common.[36,38] In China, where

home-fermented bean curd is the most common vehicle of botulism poisoning, the causative strains are a mixture of types A and B.[39] Illness is more severe and case-fatality ratios are substantially higher for type A than for type B botulism in the United States,[8,40] but are marginally higher for type B than for type A botulism in China.[39] In 1991, a large outbreak of type E botulism occurred in Egypt, traced to consumption of faseikh, a fermented fish dish eaten since the time of the Pharaohs.[41] The Republic of Georgia has the highest incidence of foodborne botulism in the world, primarily attributable to unsafe home-conservation methods of preserving vegetables (mostly type B), while smoked fish and other food products also cause some illnesses.[42] Other outbreaks outside of the United States have included type E botulism due to smoked vacuum-packed whitefish in Finland and Germany,[43,44] type A botulism due to bamboo shoots in three separate outbreaks in Thailand,[45,46] food items served at a barbeque in Austria,[47] roasted canned mushrooms in Turkey,[48] type A botulism due to commercially produced tinned fish in tomato sauce in South Africa,[49] type A botulism due to commercial mascarpone cheese in Italy,[50] and traditional Maori home-canned tiroi made with watercress and mussels in New Zealand.[51,52]

In the United States between 1899 and 2006, 1,152 outbreaks of foodborne botulism involving 2,672 persons were reported. The mean annual incidence of reported foodborne botulism in the United States in 1997–2006 was 0.1 cases per 1,000,000 persons. Generally, the northern and western states in the contiguous United States have had higher rates of foodborne botulism than the southern and eastern states. Alaska had the most cases (76) between 1997 and 2006 and had by far the highest rate of reported botulism in the United States (12 cases per 1,000,000 population).[53]

Since botulism in adults most commonly results from eating improperly preserved home-canned (or home-bottled) foods, outbreaks usually occur in family groups and affect small groups of people. In recent years, however, novel vehicles of foodborne botulism in the United States have been reported. These differ in being "fresh" rather than preserved food, and include potato salad,[54,55] baked potatoes,[56,57] sautéed onions,[58] commercial potpies,[59] turkey loaves,[56] beef stew,[56] contraband alcoholic beverage prepared by prison inmates,[60] tea made from peyote[61] and fermented tofu.[62] In each case, these unpreserved foods were prepared by ordinary cooking procedures (which do not kill *C. botulinum* spores) and then stored for many hours to days under relatively anaerobic conditions (e.g., baked potatoes in foil, sautéed onions under melted butter) at temperatures warm enough to encourage the outgrowth of spores and to support toxin production. The foods were then eaten without having been thoroughly reheated to a temperature that could destroy the heat-labile toxin. Homemade foods accounted for 67% of foodborne botulism events in 1990–2000, of which 69% were home-canned foods.[63]

Food that is commercially prepared or distributed may cause illness in a large number of persons who may be spread over a wide geographic area.[7,55,64–66] Since 1976, large outbreaks of botulism have increasingly occurred in association with foods served in restaurants; although such outbreaks constituted only 4% of botulism outbreaks in 1976–1984, these outbreaks accounted for 42% of all foodborne cases of botulism.[67] From 1990 to 2000, restaurant-prepared foods accounted for 22% of botulism outbreaks and affected 16% of all foodborne botulism cases.[63] Commercially prepared and distributed chili sauce has been implicated in two outbreaks in the United States. One occurred in 2003 and involved 15 cases in Texas.[64] This outbreak was the first outbreak that implicated food improperly refrigerated and stored at a salvage store. The other chili sauce outbreak occurred in 2007 and involved eight cases in Indiana, Ohio, and Texas.[66] This outbreak was caused by deficiencies in the commercial retort canning process, resulting in approximately 111 million cans recalled in 2007 (until this outbreak, there were no reported cases associated with commercial canning in the United States in over 30 years and this outbreak was the largest US botulism outbreak associated with commercial canning since 1919). A novel food vehicle, carrot juice, was the cause of an international outbreak involving six cases in Florida; Georgia; and Ontario, Canada in 2006. It was likely that the commercial carrot juice was inadequately refrigerated either during transport, storage by the retailer, or storage by the patients.[65] Such large outbreaks of botulism often result in extraordinary economic costs as well as extensive morbidity.[68,69] Long-term negative health effects of botulism illness have been documented.[69–74] In a case–control study conducted in the Republic of Georgia between 1998 and 2003, cases (mostly foodborne, type B botulism) after a median of 4.3 years after their botulism illness were more likely than controls to suffer from fatigue, weakness, dizziness, dry mouth, difficulty breathing while walking or lifting heavy items, and experiencing other physical or emotional problems interfering with normal activities.[72]

Although foodborne botulism affects all age groups, it occurs mainly in persons aged 30–60 years.[63,75,76] This age distribution may reflect that of persons who eat home-canned foods. Although it has been hypothesized that younger persons may be less susceptible to botulism, evidence indicates that the lower case-fatality ratios for the younger age groups are due to fewer complications during intensive respiratory support rather than to inherent resistance to the effects of botulinum toxin.[77] The disease occurs with nearly equal frequency in men and women. Rates of botulism cases reported to CDC are shown in Figure 1.

Corresponding to the distribution of botulinum spores in soil samples,[78] between 1985 and 1994, 80% of botulism outbreaks that occurred in the western United States (excluding Alaska) were type A and 19% were type B, compared with outbreaks east of the Mississippi River, where 65% of outbreaks were due to type A and 30% were due to type B. In Alaska, approximately 89% of outbreaks were of type E botulism during this period; outbreaks in Alaska have uniformly been due to fermented or otherwise preserved fish or aquatic mammals.[63,79]

5.2. Infant Botulism

Since 1980, infant botulism is the most common form of botulism reported in the United States. It is epidemiologically distinct from foodborne botulism, representing the effect not of ingestion of toxin preformed in contaminated foods, but of colonization (infection) of the intestine by spores of *C. botulinum*, with subsequent in vivo toxin production.[80] Although infant botulism was first described in 1976,[81,82] earlier cases have been identified retrospectively, and its detection probably reflects advances in diagnostic capabilities rather than the emergence of a new clinical syndrome. Cases have been reported in the United States, Australia, Argentina, Canada, Chile, Czechoslovakia, France, Great Britain, Italy, Japan, Spain, Sweden, Switzerland, and Taiwan.[83]

In the United States, 2,382 cases were reported from 50 states to CDC between 1976 and 2006 (Figure 2). Since reporting began to stabilize in 1980, the average annual incidence of reported infant botulism in the United States has been approximately 1.9 in 100,000 live births. Since 1985, 46% of all infant botulism cases have been reported from California. The incidence is highest in Delaware, Hawaii, Utah, and California (9.0, 8.8, 6.3, and 5.7 per 100,000 live births, respectively), perhaps reflecting better diagnosis and reporting in those states.

The illness is confirmed by demonstrating botulinum toxin in and isolating *C. botulinum* from the infant's stool. Toxin may be detected in serum (13% of tested infants), more often in type A cases (36%) than in type B cases (2%).[84] In the United States, between 1997 and 2006, 41% of infant botulism cases were caused by *C. botulinum* type A and 57% by type B. Seven infant botulism cases in the United States were associated with type F toxin and caused by *C. baratii* (CDC, unpublished data). Two cases in Italy were associated with type E toxin and caused by *C. butyricum*.[21] *C. botulinum* isolates producing dual toxin types have been identified in botulism cases.[85,86] Infant botulism is first seen most commonly in the second month of life and occurs somewhat earlier in cases of type B disease (median 9 weeks) than in cases of type A disease (median 11 weeks). There is no sex predilection and no apparent pattern of seasonal variation.

Infants hospitalized with the disease tend to have had higher birthweights than other infants, and their mothers tend to be white, older, and better educated than mothers in the general population. Affected infants are also more commonly breast-fed[87,88]; breast-feeding is associated with an older age at onset in type B cases.[88] Clustering of cases of infant botulism has been noted in some suburban areas in the eastern United States and in small towns and rural areas of apparent high incidence of infant botulism in the West.[89–91]

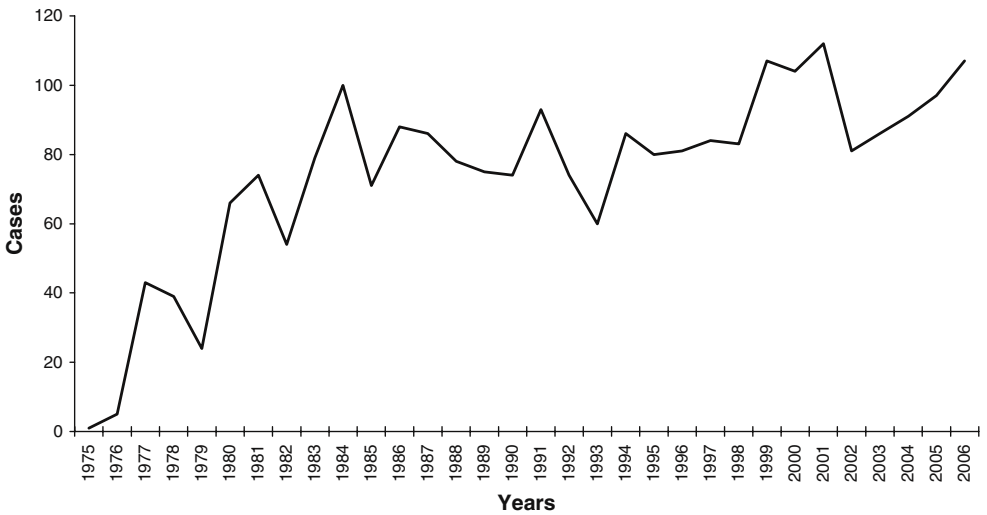


Figure 2. Cases of infant botulism reported to the Centers for Disease control and Prevention, United States, 1976–2006.

Evidence of infant botulism was detected in several cases of sudden infant death syndrome (SIDS) in Argentina, Australia, Finland, Germany, Italy, and the United States.[87,91–98] The similar age distribution of infant botulism and SIDS contributed to the hypothesis that unrecognized infant botulism might be an important cause of SIDS. One such case was an 11-week-old patient in Finland whose death was classified as SIDS and may have been a case of infant botulism.[92] *C. botulinum* isolates from the patient's intestinal contents and from his household's vacuum cleaner dust bag were confirmed to produce botulinum toxin with a mouse bioassay. In addition, two molecular typing methods, pulsed-field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD), could not distinguish the clinical specimen isolate from the vacuum cleaner dust bag isolate.

5.3. Wound Botulism

Wound botulism is a rare disease resulting from the growth of *C. botulinum* spores in a contaminated wound with in vivo toxin production.[99] Between 1943, when the syndrome was first recognized, and 2006, 390 cases of wound botulism were reported in the United States. Of 382 laboratory-confirmed cases in the United States from 1973 to 2006, 337 (88%) were type A, 33 (9%) type B, 1 (<1%) a mixture of type A and type B organisms, and 11 (3%) had serum quantity insufficient to do toxin-specific neutralization.[60] The median age of the patients was 41 years (range 5–67 years); 70% were male. The case-fatality rate was 4%. The wounds were usually deep and contained avascular areas; many patients had compound fractures, and four had extensive crush injuries of the hand. The median incubation period in cases of trauma was 7 days (range 4–21 days).[100]

The first wound botulism case associated with injection drug use occurred in a New York City patient, who attempted to inject cocaine intravenously in 1981.[101] Since then, wound botulism has dramatically increased over the past two decades largely due to cases associated with injection drug use; these cases have been associated either with needle puncture sites or with nasal or sinus lesions due to chronic cocaine sniffing.[102–104] Between 1973 and 1989, the average number of wound botulism cases in the United States was two cases per year; 28% of cases were due to injection drug use. Between 1990 and 2006, the average number of wound botulism cases increased 10-fold to 20 cases per year; nearly all (98%) were due to injection drug use (CDC, unpublished data). In some cases, as in wound botulism cases caused by other sources such as crush injuries, the wounds may not be obvious or grossly infected. Chronic drug users may have multiple superficial wound infections or abscesses from injecting several times a day, making laboratory confirmation, which entails culture of the organism from wound, difficult. The major risk factors appear to be the practice of subcutaneous or intramuscular injection ("skin-popping") of black-tar heroin and race.[103] Since the late 1980s, black-tar heroin has become the predominant cause of wound botulism west of the Mississippi River in the United States. The production of black-tar heroin involves the use of adulterants such as ground paper fiber soaked in black shoe polish and possibly dirt, which may introduce contamination with *C. botulinum* spores. Heroin-associated wound botulism cases have also been documented in Europe since 2000.[105,106]

5.4. Child or Adult Botulism from Intestinal Colonization

Isolated cases of botulism in which extensive investigation failed to implicate a specific food as the cause of the disease have been recorded by CDC since 1978 as cases of "undetermined origin" rather than of foodborne botulism. Since 1978, there have been 49 such cases reported in the United States. Although there has been speculation on the matter since the 1920s, careful investigation has now demonstrated that some of these cases are caused by colonization of the gastrointestinal tract by *C. botulinum* and *C. baratii* with in vivo production of toxin, analogous to the pathogenesis of infant botulism.[2,107] Support for the diagnosis of botulism from intestinal colonization is provided by the demonstration of the prolonged excretion of toxin and *C. botulinum* in the stool. Definitive diagnosis of this form of botulism is difficult and in most cases relies on the absence of association with a botulinum toxin contaminated food. In some cases of botulism strongly suspected of representing intestinal colonization, the patients had preceding gastrointestinal surgery or illnesses, such as inflammatory bowel disease, that may have predisposed them to enteric colonization.[2] No other specific risk factors have been identified.

5.5. Iatrogenic Botulism

Iatrogenic botulism is caused by injection of botulinum toxin at high enough doses to cause systemic disease during the treatment of cosmetic or neurological disorders. In the United States, Botox® Cosmetic (botulinum toxin type A) is licensed and indicated for the treatment of blepharospasm, strabismus, cervical dystonia, severe primary axillary hyperhydrosis, and for the temporary improvement in the appear-

ance of moderate to severe facial frown lines. Myobloc® (botulinum toxin type B) is also approved in the United States and is indicated for the treatment of adults with cervical dystonia. Off-label use of botulinum toxin for the treatment of other conditions also occurs. In 2004, an outbreak of four botulism cases occurred in the United States and involved an unlicensed physician injecting himself and three others with a research-grade botulinum toxin type A product (unlicensed for cosmetic/human use), exposing each patient to as much as 21–43 times the estimated human lethal dose by injection.[108,109] The toxin was labeled as a laboratory research product, not licensed or intended for human use, and was ordered and shipped from a legitimate manufacturer. All four patients survived; one patient, who was followed up 10 months after hospitalization, reported persistent myalgias, muscle weakness, shortness of breath, and persistent pain that required treating with an analgesic medication.[104] Botulism associated with the use of botulinum toxin injections for reducing spasticity and dystonia in pediatric patients with cerebral palsy have also been documented.[60,105] Botulism-related syndromes in these patients may include ptosis, diplopia, dysphagia, dysarthria, dystonia, fatigue, and generalized muscle weakness. A review of patients with reported adverse events during and following administration of licensed botulinum toxin was conducted.[111] This study reviewed 1,437 reports of adverse events from 1989 to 2003 through MedWatch, a voluntary system for reporting adverse events, product quality problems, or product use errors. Serious adverse events reported in patients treated with licensed toxin type A for therapeutic reasons (for both labeled and unlabeled indications) covered a wide variety of reactions including death, dysphagia, muscle weakness/spasm, allergic reactions, flu-like syndromes, gastrointestinal symptoms, cardiovascular-system related symptoms, respiratory system-related symptoms, and seizure. No deaths were reported in patients treated with toxin type A for cosmetic purposes, but commonly reported reactions included headaches, focal facial paralysis, muscle weakness, dysphagia, flu-like syndromes, and allergic reactions.

6. Mechanisms and Routes of Transmission

6.1. Foodborne Botulism

Foodborne botulism is caused by consuming improperly preserved food in which spores of *C. botulinum* have germinated and organisms have grown and produced toxin. Home-processed foods accounted for 95% of foodborne botulism outbreaks in the continental United States in the past decade. Mass-processed commercial foods accounted for only 5% of outbreaks and 3% of cases. Even the large, restaurant-associated outbreaks of recent years have generally been caused by foods either home-canned in the restaurant[112] or otherwise improperly handled or stored,[7,58] rather than by defective commercially canned products.

Temperatures obtainable only with a pressure cooker are usually necessary to kill *C. botulinum* spores.[25,113] Boiling alone does not kill the spores of proteolytic strains, but does kill competing organisms and creates an anaerobic environment that will support the growth of *C. botulinum* once the spores germinate. The toxin itself is heat-labile; heating to 80°C for 10 min is sufficient to destroy the toxin. However, some home-canned foods are not cooked before being eaten, and those that are cooked may not be subjected to sufficient heat to destroy all the toxin present.

Although the epidemiological investigation of outbreaks has improved and the vehicles of botulism outbreaks have been more frequently identified in recent years, the proportional contribution of different types of food to cases of foodborne botulism has remained relatively stable (Table 1). Vegetables and fish/aquatic animals have continued to be the most common food vehicles in foodborne cases over the years. Vegetables, especially those of more neutral pH such as asparagus, green beans, peppers, and mushrooms, are responsible for most type A and type B outbreaks. Spores may survive when either hot-pack or cold-pack canning methods are used[113]; cold-pack canning is acceptable only for certain fruits because of their high acidity or low water activity due to their high sugar content, which inhibits outgrowth of *C. botulinum* spores. Type E outbreaks are most often caused by products derived from fish and marine mammals. For example, many type E outbreaks in Alaska are associated with "muktuk" and "stink eggs," in which whale blubber or fish eggs are fermented in an anaerobic milieu, allowing the outgrowth of *C. botulinum* organisms.[78,114]

Table 1. Vehicles of Foodborne Botulism in the United States, 1899–1977 (1961 Cases), 1978–1985 (235 Cases), 1986–1994 (227 Cases), and 1995–2006 (249 Cases)*

	Percent of total cases			
Vehicle (number of cases)	1899–1977 (1961)	1978–1985 (235)	1986–1994 (227)	1995–2006 (249)
Unknown	65.5	3.0	16.0	18.0
Identified	34.5	97.0	84.0	81.9
Vegetables	57.0	64.0	45.0	33.2
Fish/aquatic animals	15.6	19.3	45.0	45.8
Fruits	11.0	0.9	0	1.1
Condiments	8.7	10.1	4.7	1.6
Meat/dairy	7.5	5.7	3.7	18.4

* Data for 1899–1994 from Angulo FJ, St Louis ME. Botulism. *Bacterial Infections of Humans: Epidemiology and Control*. 3rd ed. New York: Plenum Medical Book Co., 1998.

6.2. Infant, Wound, and Child or Adult Botulism from Intestinal Colonization

In contrast to foodborne botulism, which is an intoxication, infant, wound, and child or adult botulism from intestinal colonization results from colonization by neurotoxin producing *Clostridia* species. These organisms are widely distributed in nature and can easily be ingested with foods[78,115] or contaminate a traumatic wound. The low frequency of *C. botulinum* infections despite the ubiquity of the organism in nature suggests that host factors are prominent in the epidemiology of these infectious forms of botulism.

Infant botulism results after colonization of the infant intestine.[80] In a prototypical case, type B organisms, but no toxin, were isolated from honey fed to an infant with infant botulism whose fecal specimens contained type B organisms and toxin. Family members who ate some of the same honey did not become ill. In several studies from the late 1970s and early 1980s, more than 20% of affected infants had ingested honey before the onset of botulism.[88,116,117] In many cases, *C. botulinum* spores of the same type were cultured from honey in the same households.

However, since most infants with infant botulism have had no exposure to honey, the risk factors and vehicles of transmission of *C. botulinum* for the majority of cases remain unclear.[80,118] A survey of foods commonly fed to infants revealed *C. botulinum* in specimens of corn syrup as well as honey, but in no other tested category of foods.[115] In other studies, the same types of *C. botulinum* that caused disease were isolated from soil in an infant's yard and from vacuum cleaner dust; investigators have also frequently noted environmental conditions that might expose infants directly to environmental sources of *C. botulinum* spores, such as a shared crib, dusty or windy locales, nearby building construction, or outdoor activities.[89,91] Infants hospitalized with botulism have also more typically been breast-fed than have control infants.[87,118–120] Breast-feeding is known to affect the fecal flora differently than formula feeding; in mice, the fecal flora have been shown to be an important susceptibility factor in challenge experiments with *C. botulinum* type A spores.[121]

Wound botulism, like tetanus, gas gangrene, and other clostridial diseases, can result from soil contamination of a wound.[100] Although the diagnosis of wound botulism may be frequently missed, the extreme rarity of wound botulism despite the wide distribution of the organism in the environment raises many questions regarding its pathogenesis: Are many wounds contaminated but few suitable for proliferation of the organism? Or is wound contamination with *C. botulinum* without signs of botulism truly uncommon? Data are scarce. Part of the problem is the difficulty in obtaining adequate anaerobic cultures and in differentiating *C. sporogenes* (a common wound isolate) from *C. botulinum*. In addition, the factors involved in spore germination, growth, and toxin production in tissues or in vivo systems have not been well studied.

In cases of adult botulism in which intestinal colonization was proven or suspected, vehicles have included canned cream of coconut[107] and blackberry preserves,[122] in each of which *C. botulinum* organisms of the appropriate type were detected. In a case strongly suggestive of this syndrome, a healthy, 27-year-old asymptomatic consumer of blood sausage was shown to have intestinal carriage of *C. botulinum* 19 days after an outbreak attributed to the sausage, but did not develop clinical symptoms and botulinum toxemia until 47 days after the outbreak.[2] The limited data about enteric infectious botulism in adults do not suggest that the vehicles of transmission are categorically different from those of traditional foodborne botulism due to preformed toxin.

7. Pathogenesis and Immunity

Incubation periods for foodborne botulism are reported to be as short as 6 h or as long as 10 days,[7] but generally the time between toxin ingestion and onset of symptoms ranges from 18 to 36 h.[40] Among patients with foodborne botulism, incubation periods are shorter for patients with illness from type E toxin.[40] The incubation periods for wound botulism (4–14 days) are longer than those usually seen in cases of foodborne botulism, and presumably reflect the time required for the multiplication of *C. botulinum* in the wound and the release of toxin. It has been shown that those individuals with shorter incubation periods—apparently those who ingest and/or absorb larger amounts of toxin—have more severe disease and a graver prognosis.[123] The incubation period has not been determined for cases of infant botulism.

Botulinum toxin is produced by the bacterial cell as a polypeptide chain with a molecular weight of 150 kDa (100 kDa heavy chain and 50 kDa light chain) and is noncovalently associated with other proteins (hemagglutinin, HA, and nontoxin nonhemagglutinin, NTNH). The complex is absorbed into the bloodstream from the gastrointestinal tract likely through a receptor-mediated endocytosis process. The complex disassociates in the bloodstream and botulinum toxin is transported via lymphatics and blood to cholinergic neuromuscular junctions. The mechanism for transport of botulinum toxin into the extracellular is not known. But here the toxin, through the heavy chain, is fixed at and then the light chain is internalized into the presynaptic nerve endings through receptor-mediated endocytosis and pH induced

translocation. The light chain, which is unique for each of the 7 toxin types, is a zinc-endopeptidase which cleaves a specific peptide sequence of one of several proteins involved in the release of acetylcholine from the nerve ending.[124] Blockade of acetylcholine release prevents muscle contraction and results in flaccid paralysis that is characteristic of botulism[125,126]. Evidence suggests that the binding of botulinum neurotoxin to the nerve ending is irreversible[126]; hence, functional recovery occurs only with the regeneration of neuronal fibrils and the reestablishment of the neuromuscular junction. The length of action of botulinum toxin may also be due to the internal half life of toxin or may be due to some unknown features specific to the protein substrates of the light chain. And these variables may be toxin-type dependent. This may explain why muscular weakness persists in many individuals for weeks or more after toxin has been eliminated from the intestine and bloodstream and why in severely affected individuals recovery may take many months to years or may never be complete.[58,71] Observation of patients has shown that patients with type A botulism (foodborne or infant) take longer to recover than those with type B or type E botulism. Differences in light chain stability and/or differences in recovery of protein substrate may explain the toxin type -dependent recovery rate in patients.

Botulinum toxin affects acetylcholine release only at peripheral sites; the central nervous system is not affected. Botulinum toxin does not appear to have the ability to cross the blood-brain barrier, but in vitro studies have shown that tissues of central nervous system origin are affected by the toxin's action.[124] Both the autonomic and voluntary motor activities of the cranial nerves seem to be uniquely susceptible to the effects of botulinum toxin. The cranial nerves are nearly always affected earlier and to a greater degree than are the nerves to the peripheral and respiratory muscles. Certain cranial nerves seem to be more sensitive to one botulinum toxin type than to others. Pupillary paralysis, for example, is apparently more commonly noted in type A botulism than in type B botulism.[40,71,77] Correspondingly, distinctions in the effect of type A and B toxin on laboratory preparations of the neuromuscular junction have been observed.[127] Although the reason for this is not clear, molecular differences between toxin types that account for immunologic distinction might, through stearic interactions, also result in disparate binding capabilities at neuromuscular junctions.

Botulinum toxin is typically so potent that the lethal dose is far below that required to induce an antibody response. In one case of adult intestinal colonization, protective antibodies developed and remained at a protective level for more than a year.[128]

Animal models of infant botulism have yielded insight into mechanisms of intestinal colonization and in vivo growth of *C. botulinum*. Successful intraintestinal colonization by *C. botulinum* spores inoculated into the stomachs of infant mice is strongly age-dependent, peaking at 7–13 days old, a period during which the enteric microbial flora are in transition.[129] Although normal adult mice are highly resistant to colonization, germ-free adult mice may develop botulism after ingesting as few as ten spores,[130] and antimicrobial treatment of normal adult mice dramatically lowers the dose of ingested spores necessary to yield intraluminal outgrowth and production of toxin.[131] In mice, therefore, enteric infection is limited to a narrow age range in infancy and to occurrence in adults that have undergone a manipulation of the normal intestinal microbial flora. It is possible that anatomic variations, stasis, or constipation due to mechanical or motility factors or some combination of these contribute to the creation of an environment favorable to the growth of the organism.

Factors other than just the physical production of toxin in the gut may also contribute to the expression of clinical illness. *C. botulinum* organisms and toxin have been recovered from the feces of human infants more than 8 weeks after the onset of botulism, and the peak excretion of toxin may not occur until several weeks after the illness has already begun to resolve.

8. Patterns of Host Response

8.1. Clinical Features

The clinical syndrome of botulism, whether foodborne, infant, wound, or adult or child intestinal colonization, is dominated by the neurological symptoms and signs resulting from a toxin-induced blockade of the voluntary motor and autonomic cholinergic junctions and is essentially quite similar for each syndrome and toxin type.[77,80,100,120] The ingestion of other bacteria and their toxins in the improperly preserved food may account for the abdominal pain, nausea and vomiting, and diarrhea that often precede or accompany the neurological symptoms of foodborne botulism. Dryness of the mouth, inability to focus to a near point (prompting the patient to complain of "blurred vision"), and diplopia are usually the earliest neurological complaints. If the disease is mild, no other symptoms may develop and the initial symptoms will gradually resolve. The person with mild botulism may not come to medical attention. In more severe cases, however, these initial symptoms may be followed by dysphonia, dysarthria, dysphagia, and peripheral muscle weakness. If illness is severe, respiratory muscles are involved, leading to ventilatory failure and death unless supportive care is provided. Patients have required ventilatory support for up to 7 months before the return of muscular function, although a 2- to 8-week duration of ventilatory support is more common.[77] Death occurs in 5–10% of cases of food-

borne botulism; early deaths result from a failure to recognize the severity of disease or from pulmonary or systemic infections, whereas deaths after 2 weeks are from the complications of long-term mechanical ventilatory management or respiratory malfunction.[77] An example of how a rapid response to a public health emergency can prevent deaths can be seen in a large foodborne outbreak in Thailand in 2006, in which no deaths occurred among the 209 patients affected. Early detection, prompt administration of antitoxin, supportive care of patients, and collaboration of public health resources in Thailand and globally contributed to the success of the response efforts to this public health emergency.[133]

Perhaps because infants are not able to complain about the early effects of botulinum intoxication, the neurological dysfunction associated with infant botulism often seems to develop suddenly. The major manifestations are poor feeding, diminished suckling and crying ability, neck and peripheral weakness (the infants are often admitted as "floppy babies"), and ventilatory failure.[80,87,120] Constipation is also often seen in infants with botulism and, in some, has preceded by many days the onset of neurological abnormalities. Loss of facial expression, extraocular muscle paralysis, dilated pupils, and depression of deep tendon reflexes have been reported more frequently with type B than with type A infant botulism.[120] Treatment with aminoglycoside antimicrobial agents may potentiate neuromuscular weakness in infant botulism[133] and has been associated with an increased likelihood of needing mechanical ventilation.[80,120] The median length of hospital stay in cases of infant botulism cases treated with human immunoglobulin is 2.6 weeks (range, 2.0–3.3 weeks).[134] Fewer than 2% of reported cases of infant botulism result in death.

8.2. Diagnosis

Botulism is probably substantially underdiagnosed. The diagnosis is not difficult when it is strongly suspected, as in the setting of a large outbreak. However, since cases of botulism most often occur singly, the diagnosis may pose a more perplexing problem. Findings from many outbreaks have suggested that early cases are commonly misdiagnosed and may be diagnosed only retrospectively after death, after the subsequent clustering of cases of botulism-like illness finally alerts public health personnel to the occurrence of an outbreak of botulism[5,6,135]; other cases are undoubtedly missed entirely. Entire outbreaks may even go undetected despite severe illness in patients; for example, one outbreak was recognized retrospectively only after a second cluster of cases occurred due to the same vehicle.[7] In this particular outbreak, two sisters diagnosed with botulism led to the identification of 36 unrecognized cases of type B botulism in the preceding 6 weeks.

Botulism should be suspected in any adult with a history of gastrointestinal, autonomic (e.g., dry mouth, difficulty focusing), and cranial nerve (diplopia, dysarthria, dysphagia) dysfunction or in any infant with poor feeding, diminished sucking and crying ability, neck and peripheral muscle weakness, and/or ventilatory distress.[8,136] The demonstration of bilateral cranial nerve findings and the documentation of neurological progression (peripheral muscle weakness, ventilatory compromise) increase the level of suspicion. The diagnosis is even more likely if an adult patient has recently eaten home-canned foods or if family members are similarly ill, or both. If the typical clinical syndrome is present and no food item can be pinpointed as a means of transmission, a contaminated wound should be sought. If the typical syndrome is seen and a wound is identified, the wound should be explored and, in addition to routine cultures, specimens should be taken for anaerobic culture and toxicity testing even if the wound appears clean.

The differential diagnosis includes myasthenia gravis, stroke, Guillain–Barré syndrome, bacterial and chemical food poisoning, tick paralysis, chemical intoxication (e.g., from carbon monoxide, barium carbonate, methyl chloride, methyl alcohol, organic phosphorus compound, or atropine), mushroom poisoning, medication reactions (e.g., from antibiotics such as neomycin, streptomycin, kanamycin, or gentamicin), poliomyelitis, diphtheria, and psychiatric illness. In infant botulism, sepsis (especially meningitis), electrolyte–mineral imbalance, metabolic encephalopathy, Reye's syndrome, Werdnig–Hoffman disease, congenital myopathy, and Leigh's disease should also be considered.

Routine laboratory studies are not helpful in confirming the clinical suspicion of botulism. Serum electrolytes, renal and liver function tests, complete blood tests, urinalysis, and electrocardiograms will all be normal unless secondary complications occur. A normal cerebrospinal fluid (CSF) examination helps differentiate botulism from Guillain–Barré syndrome, except protein may be normal early in the course of Guillain–Barré and a slightly elevated CSF protein level is occasionally seen with botulism.[77] Normal neuroradiological studies, such as computed tomographic scans or magnetic resonance imaging, help to rule out stroke, another condition commonly confused with botulism.[7,135] Although, early in the course of a potential cerebral vascular event, neurological studies may be normal.

Electromyography (EMG) may be helpful in distinguishing botulism from myasthenia gravis and Guillain–Barré syndrome, diseases that botulism often mimics closely. A characteristic EMG pattern observed in adult patients with botulism has been well described[71,137,138] and includes a low-amplitude response to a single stimulus, a decremental trend in amplitude of the elicited action potentials

to repetitive low-frequency stimulation (2–5 Hz), and an incremental trend (facilitation) to rapid repetitive stimulation (20–50 Hz). However, rapid repetitive stimulation is much more sensitive and specific for botulism than low-frequency stimulation.[7] EMGs should be performed on clinically involved muscles; positive results may be obtained from only one muscle even though many are weak.

The most prominent EMG finding is infant botulism is the same as that in adults: an incremental response to rapid repetitive stimulation.[139] However, another EMG pattern has been described as characteristic of infant botulism.[140] This is the pattern of "brief duration, small amplitude, overly abundant (for the amount of power being exerted)" motor unit action potentials, termed "BSAP" (brief-duration, small-amplitude, overly-abundant motor-unit action potentials) by Engel.[141] Polyphasicity may also be part of this pattern. It is not unusual to find either a BAP pattern (if the motor unit potentials are only brief) or a SAP pattern (if the motor unit potentials are only small) in patients who have typical BSAP findings in other parts of a muscle or in other muscles.

Detection of toxin in serum provides laboratory confirmation of a case of botulism (other than infant). Serum from infants is rarely positive and is difficult to obtain from those most at risk due to their young age. In addition to serum testing, culture of tissues debrided from a wound may facilitate laboratory confirmation of wound botulism cases. Toxin detection in stool specimens or epidemiologically incriminated foods and/or toxin production in culture of stool can be used to confirm a case of foodborne botulism.[8,9] Cultures were positive for 51% of stool specimens and toxin testing was positive for 37% of sera and 23% of stool specimens collected from 309 persons with clinically diagnosed botulism reported to CDC from 1975 to 1988; at least one laboratory test was positive for 65% of patients.[40] Collecting stool and sera samples early during the course of illness increases the likelihood of obtaining positive results. However, in any given situation, these tests may not be helpful; large outbreaks have occurred in which none[142] or a very low percentage[149] of specimens yielded positive results. In addition, laboratory results may not be reported until many hours or days after the specimens are received. The administration of antitoxin is the only specific therapy available for botulism, and evidence suggests that it is effective only if given very early in the course of neurological dysfunction.[123] Hence, the diagnosis of this illness cannot await the results of studies that may be long delayed and may be confirmatory only in some cases. The diagnosis should be made on the basis of the history and physical findings.

9. Prevention, Control, and Treatment

9.1. Prevention and Control

The prevention of foodborne botulism depends on destroying all *C. botulinum* spores in food as it is preserved or creating a milieu in the preserved foods that will not allow the growth of any organisms that survive preservation procedures. This requires careful home canning and commercial canning techniques. Several private and government agencies (e.g., the US Department of Agriculture) provide information on the proper methods of home canning. Although the heat resistance of *C. botulinum* spores is quite variable, cooking at 121°C (250°F) or higher is usually sufficient to destroy all spores.[25–27] These temperatures can be achieved only in a pressure cooker. Boiling alone may kill competitive organisms and create an anaerobic environment highly favorable to the growth of *C. botulinum*. Boiling is sufficient for fruits and certain vegetables, especially those with added sugar, because their high sugar content will not support the growth of *C. botulinum*.[24] Commercial chopped garlic bottled under oil has caused two outbreaks of botulism.[7,114] As a result, this food must now be acidified with phosphoric acid. Previously, tomatoes were thought to be an extremely low-risk food because of their acidity. Cases of botulism, however, have been caused by contaminated foods that contained tomatoes (such as spaghetti sauce) and by tomato juice.[76] These cases have been attributed to the low-acidity tomatoes that are now being bred for increased "sweetness." Adequate refrigeration alone may be the only control method in the absence of other factors that prevent the germination of *C. botulinum* spores and toxin production in some products, as seen in an outbreak traced to carrot juice, in which the product was not intended to be re-heated before consumption.[65]

Home-canned foods that are contaminated with proteolytic *C. botulinum* organisms or concurrently contaminated with other anaerobic or aerobic organisms will spoil. Foods that look or smell spoiled should not be tasted. Foods contaminated by nonproteolytic strains of *C. botulinum* (and sometimes proteolytic strains as well) may not appear spoiled. Nonproteolytic strains, including type E, may continue to grow even at 3.3°C with little competition from other spoilage bacteria. Food items suspected of having transmitted botulism should be carefully sealed and marked and then be refrigerated and saved for culture and toxin testing. Although *C. botulinum* spores are resistant to heat, botulinum toxins are heat-labile and are readily destroyed by boiling. Thorough boiling of home-canned foods is an added safeguard against botulism.

Commercial canners use a variety of methods to prevent the growth of *C. botulinum*. Commercial canners routinely heat foods to 120°C for 3 min, and often acidify high-risk, low-acid foods such as peppers to inhibit the growth of *C. botulinum*. Semipreserved meat products (e.g., hot dogs and luncheon meats) have nitrites added for similar reasons. Systems for killing spores in foods by radiation are also under investigation. *C. botulinum* is a gas-producing organism, and its growth inside a can may produce enough gas to make the can bulge or swell. More common causes of bulging include overpacking by the manufacturer, gas caused by interaction of the contents and the metal container, or contamination by some other organisms due to a fractured seal. Nevertheless, swollen, bulging cans or cans with spoiled contents should not be consumed and should be reported to local health authorities or to the US Food and Drug Administration (FDA).

Physicians also have an important role in the control of botulism. All suspect cases of foodborne botulism should be reported immediately to local or state health authorities or directly to CDC in Atlanta (telephone 770-488-7100); CDC can assist with clinical consultation, laboratory testing, and antitoxin release. It is important that this be done quickly and efficiently, especially if the suspected vehicle is a commercial product. If the diagnosis is suspected, a search for other cases (initially by identifying persons who shared the implicated food) should be immediately undertaken.

Because the mechanism of transmission for infant botulism has not been totally defined, few control measures are available. Controlled epidemiological studies will be required to develop the necessary data. Since honey is the one identified source of *C. botulinum* spores for susceptible infants and since it is not essential for good infant nutrition, it is recommended that honey not be fed to infants under age 1 year.[80] The prevention of wound botulism depends on the thorough cleansing and debridement of wounds, especially those of any depth or that are contaminated by soil. Wound botulism may be prevented by avoiding the use of black-tar heroin.

Botulinum toxoid, as an investigational new drug (IND) has limited availability.[145] However, because of the rarity of botulism in the general population and the side effects of immunization, toxoid administration is recommended only for laboratory personnel working directly with the organism and the toxin. The toxoid is available from CDC Drug Service (404-639-3670).

9.2. Treatment

The mainstays of treatment of foodborne and wound botulism are as follows: (1) administration of botulinum antitoxin in an attempt to prevent neurological progression of a moderate, slowly progressive illness or to shorten the duration of ventilatory failure in those with a severe, rapidly progressive illness; (2) careful monitoring of respiratory vital capacity, aggressive respiratory care, and mechanical ventilation of needed for those with ventilatory insufficiency; and (3) meticulous and intensive care for the duration of the often prolonged paralytic illness.

Antitoxin therapy is more effective if undertaken early in the course of illness and may reduce the duration of mechanical ventilation shorten the course of illness, and increase the chance of survival.[45,123,146] This is not surprising when one considers that equine antitoxin neutralizes only toxin molecules yet unbound to nerve endings.[126] Most persons (more than 80%) with adult botulism in the United States are treated with equine antitoxin. Administration of one vial of bivalent (types A and B) and one vial of type E botulism antitoxin by the intravenous route results in serum levels of type A, B, and E antibodies capable of neutralizing serum toxin concentrations manyfold in excess of those reported for botulism patients. Therefore, administration of one vial of antitoxin intravenously is recommended and antitoxin need not be repeated, since the circulating antitoxins have a half-life of 5–8 days.[147] However, treatment is not without risk as approximately 9.0% of persons treated experience hypersensitivity reactions, although these data were from the period when more than one vial was given and it is possible that the percentage of persons experiencing hypersensitivity reactions is lower given administration of one vial.[148] It is therefore extremely important that physicians recognize botulism as early in its course as possible, and yet not mistake other neurological syndromes for botulism. CDC has a 24/7 consultancy and antitoxin release service for physicians through state health officials and can be contacted by telephone, 770-488-7100. A heptavalent (types A, B, C, D, E, F, and G) botulinum antitoxin product is currently not licensed in the United States, but would be provided by CDC to treat patients in an emergency situation or those with unusual suspected types C, D, E, F, or G botulism under a treatment investigational new drug (IND) application. In the future, this heptavalent botulinum antitoxin product may become licensed in the United States and in the meantime may be utilized if existing botulinum antitoxin products are unavailable.

For infant botulism, treatment with antibiotics is not indicated as aminoglycosides potentiate the paralytic effects of botulinum toxin. Treatment of infant botulism with the drug, human botulism immune globulin intravenous (BIG-IV, sold under the name of BabyBIG), has been found to be safe and effective in decreasing length of hospital stay,

hospital charges per patient, duration of intensive care unit stay, duration of mechanical ventilation, and duration of tube or intravenous feeding.[127,142] In a 5-year randomized, double-blinded placebo-control trial of BIG-IV in California, patients treated with BIG-IV had significantly shorter length of hospital stay (half), ICU stay, duration of mechanical ventilation, and tube or IV feeding compared to controls. Hospital charges per patient was on average 88,600 US dollars less for patients treated with BIG-IV compared to controls. The number of serious adverse events in patients treated with BIG-IV did not differ significantly with controls. The main adverse event that may be attributed to BIG-IV is erythematous rash.[127,142] BIG-IV was licensed by the FDA following the 5-year clinical study and a 6-year open-label study nationwide. BIG-IV is sold under the name of BabyBIG by the California Department of Health Services' Infant Botulism Treatment and Prevention Program and can be obtained by calling 510-231-7600 or visiting http://www.infantbotulism.org.

10. Bioterrorism

Botulinum neurotoxins are on CDC's list of select agents and toxins (http://www.cdc.gov/od/sap/docs/salist.pdf) as a Category A agent. Category A agents are the highest priority agents that pose a risk to national security because they can be easily disseminated or transmitted person-to-person, cause high mortality, might cause public panic and social disruption, and require special action for public health preparedness.[150] Historically, attempts have been made to use botulinum toxin as a bioterror weapon.[151,152] To date, botulism caused by inhalation of aerosolized toxin has only occurred in laboratory workers. These patients had similar neurological symptoms seen in foodborne botulism and recovered within 2 weeks following inhalational exposure. The course of illness suggested for inhalational botulism is irritant upper airway prodrome with initial exposure followed by a variable onset of different degree of paralysis in exposed patients.[157] Because of its potency, small amounts of botulinum toxin have the potential of causing widespread and catastrophic illness. The severity of illness and potential for intentional release of botulinum toxin highlight the need for clinicians to recognize the symptoms of botulism and for diligent surveillance by federal, state and local health departments. Every suspicious case of botulism, whether naturally occurring or suspected bioterrorism, should be reported to CDC's Emergency Operations Center (770-488-7100).

11. Unresolved Problems

Much is now known concerning the epidemiological and clinical aspects of adult forms of botulism. The most prominent persisting problem may be the low index of suspicion for botulism among clinicians who are unlikely to diagnose even one case in a career. The development of laboratory tests that are equally as sensitive and specific as the mouse bioassay, but more rapid, would be helpful in confirming illness and potentially could help support treatment recommendations. The development of an antitoxin derived from human serum or by monoclonal antibodies could provide an approach to antitoxin therapy that is safer (because less likely to cause hypersensitivity reactions than equine antitoxin) and perhaps less expensive. Although the clinical spectrum of illness and descriptive epidemiology of infant botulism have been well characterized in the few years since its discovery, specific risk factors and vehicles of transmission remain obscure for most cases. Controlled epidemiological studies are needed to further clarify ways of preventing this important source of childhood morbidity. The demonstration in some adults of a pathogenic mechanism similar to that of infant botulism blurs somewhat the distinction between adult and infant forms of the disease, and may initiate reconsideration of pathogenesis and treatment in both groups. Finally, increasing identification of *Clostridia* other than *C. botulinum*, in particular the rise in the occurrence of botulism due to *C. baratii* type F, has further aggravated the complexity of laboratory investigations of botulism.

All cases of suspect foodborne and wound botulism and adult botulism from intestinal colonization should be reported to CDC through state health departments. CDC can assist with clinical consultation, laboratory testing, antitoxin release, and epidemiologic investigation to identify vehicles and get implicated products removed from stores and homes quickly.

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CHAPTER 8

Brucellosis

Edward J. Young

1. Introduction

Brucellosis is an infectious disease of animals (zoonosis) that is transmittable to humans. Humans are always accidental hosts, playing no role in maintaining the disease in nature. In the United States, brucellosis has been largely controlled as a result of the Federal/State Cooperative Bovine Brucellosis Eradication Program.[1] Consequently, reports of human brucellosis have declined to about 100 cases per year, with most originating in areas where the disease remains enzootic.[2]

Although there is a high degree of relatedness among *Brucella* species, the genus is traditionally classified into six nomen species based on their major animal hosts: *Brucella abortus* (cattle), *B. melitensis* (goats and sheep), *B. suis* (swine), *B. canis* (dogs), *B. ovis* (sheep), and *B. neotomae* (desert wood rats). To this list must be added recent brucella isolates from marine mammals tentatively termed *B. maris*. *Brucella ovis* and *B. neotomae* are not human pathogens, and the potential for marine mammal strains to cause human infection remains uncertain.[3]

Animals are the only source of brucellosis and there are no known vectors. Brucellosis in humans is a protean disease characterized by a plethora of nonspecific complaints, such as fatigue, anorexia, weight loss, malaise, body aches, and depression. Aside from fever, and occasionally hepatosplenomegaly, there can be a paucity of abnormal physical findings.

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2. Historical Background

It is likely that brucellosis has existed since man first domesticated animals; however, Jeffery Allen Marston, a Royal Army Medical Corps (RAMC) surgeon, is credited with the first accurate description of the disease among troops invalided to Malta during the Crimean War.[4] David Bruce, another RAMC surgeon, researched Malta fever among military personnel at the Station Hospital in Valletta, and in 1887 isolated a gram-negative coccobacillus from the spleens of seven fatal cases. In 1897, M.L. Hughes, RAMC, published a classic monograph reviewing the clinical and pathological features of the disease and suggesting the name undulant fever.[5] The same year, Wright and Semple successfully applied the method of serum agglutination for diagnosis, enabling clinicians to differentiate brucellosis from typhoid, malaria, and other febrile illnesses.

In 1904, the Mediterranean Fever Commission was established, and between 1904 and 1907, seven *reports* were published detailing the bacteriology, epidemiology, and pathology of brucellosis.[6] In 1905, Themistocles Zammit, a civilian member of the Commission, identified Maltese goat as the animal host of brucellosis and fresh goat's milk as the vehicle of transmission from animals to human.[7] The causative organism was named *Micrococcus melitensis*, and it was found to be widespread in goat populations around the Mediterranean and elsewhere in Europe.[8] When unboiled goat's milk was eliminated from the diets of the military, the number of cases of human brucellosis declined precipitously.

In 1895, the Danish physician and veterinarian Bernhard Bang isolated an organism he termed *Bacterium abortus* from placental tissue of cattle suffering contagious abortions.[9] This disease was believed to be different from that producing abortions in goats and sheep; therefore, no human infection was ascribed to the known presence of Bang's bacillus in the milk of many herds.[10] In 1918,

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Alice Evans published data showing the antigenic relatedness between *B. melitensis*, the causative agent of Malta fever, and *B. abortus*, the agent of contagious abortion in cattle.[11] Subsequently, the genus was named *Brucella* to honor Bruce. Human infection with *B. abortus* was documented in 1924 by Orpen[12] in Great Britain, although studies by Morales-Otero using volunteers in Puerto Rico showed that *B. abortus* was more easily transmitted via direct inoculation through breaks in the skin than by ingestion of milk.[13]

In 1914 Jacob Traum isolated *B. suis* from an aborted swine fetus,[14] although the organism may well have been recovered as early as 1909 by Hutyra and Marek in Hungary.[15] *Brucella suis* has a more limited geographic distribution than either *B. abortus* or *B. melitensis*, and its biovars have different natural hosts. Biovars 1, 2, and 3 cause infection in swine, whereas, biovar 4 is limited to reindeer and caribou populations of the sub-Arctic regions of Alaska, Canada, and Siberia. Biovar 5 is a rodent pathogen and human infections have been limited to laboratory accidents.[16] Human infection with *B. suis* biovars 1–3 is notably associated with certain occupational groups, including meat inspectors, butchers, and abattoir workers. Infection with *B. suis* is also a risk for feral swine hunters, since the disease is widespread in these animals and their popularity as game animals increases. During butchering, hunters are exposed to blood and body fluids and the risk of contracting the disease is extremely high, since *B. suis* is shed continuously in urine and seminal discharges, not just at the time of abortion or parturition.[17]

Brucella canis was first reported as the cause of contagious abortion among kennel-bred dogs in 1964 by Carmichael and Bruner,[18] and human infection was documented in 1972 by Swenson and colleagues.[19] Serological studies of dogs of different breeds indicate that *B. canis* is widespread throughout the world.[20] The incidence of *B. canis* infections in humans is not known with certainty. The few cases reported in the literature indicate close contact with infected dogs or their secretions or laboratory-acquired infections.[21]

In 1994, Ross and colleagues isolated brucellae from the carcasses of marine mammals that were stranded off the coast of Scotland,[22] and Ewalt and colleagues recovered a *Brucella* species from the aborted fetus of a bottlenose dolphin in California.[23] Subsequently, several *Brucella* strains were isolated from a wide variety of marine mammal species throughout the world.[24] These strains are distinct from previously recognized nomen speciesand can be divided into two groups based on cultural characteristics and host species. The group was tentatively called *B. maris*, with putative biovars *B. cetaceae* and *B. pinnepediae* from their natural host species.[2,25]

3. Methodology

3.1. Sources of Data

In the United States, human brucellosis is a notifiable disease and morbidity and mortality data are reported weekly to the Centers for Disease Control and Prevention (CDC)[26]; however, there is evidence to suggest that underreporting remains a problem. For example, a study by the Texas Department of Health in 1990–1991 indicated that less than one half of the cases of human brucellosis diagnosed were actually reported.[27] Worldwide, information on human brucellosis is tabulated by the World Health Organization (WHO); however, since the disease is not reportable in many countries the reliability of these data is questionable. Moreover, in many countries where the disease is enzootic, facilities for diagnosis and mechanisms for reporting are limited, so numbers of cases may be under- or overestimated.

3.2. Surveys

Serological surveys for brucellosis in cattle and swine are routinely performed under the guidelines of the Brucellosis Eradication Program and states are rated according to the numbers of reactor animals in test herds and at sale barns. Rules and regulations concerning the control and eradication of the disease in animals are published periodically in the *Federal Register*.

3.3. Laboratory Diagnosis

3.3.1. Isolation and Identification of the Organism. A definitive diagnosis of is made by recovering *Brucella* from blood, bone marrow, or other tissues. Since brucellae are facultative intracellular pathogens that rapidly localize within organs of the reticuloendothelial system, cultures of bone marrow may have a higher yield than blood.[28] A variety of bacteriological methods have been used including the broth/agar technique of Castañeda; however, automated systems such as BACTEC have reduced the time of isolation from weeks to days.[29] Isolation rates for brucella bacteremia vary from 15 to 70%, in part, relating to the methods used and the intensity of the search.[30] Because brucellae grow slowly in vitro, it is necessary to maintain cultures for at least 4 weeks before declaring them negative. Polymerase chain reaction (PCR)-based assays have been developed for the identification of *Brucella* species and have found use in veterinary diagnostics.[31] Although a variety of primers have been studied, to date, PCR has found only limited use in the diagnosis of human brucellosis.[32] Nevertheless,

advances in PCR technology, such as rapid cycle real-time PCR and combinations of PCR with restriction enzymes, have made the technique more rapid and more specific.[33] Clinical laboratories utilizing automated rapid identification systems should ensure that they contain database profiles for *Brucella*, since misidentifications as *Moraxella* sp. or *Haemophilus* sp. have been reported.[34] Brucellosis has long been recognized to be a risk for laboratory personnel handling clinical material[35–37] and the use of biohazard precautions is mandatory.

3.3.2. Serological and Immunological Tests. In the absence of bacteriologic confirmation, the diagnosis of brucellosis depends on the demonstration of high or rising titers of specific antibodies in the serum. During the course of infection with *Brucella* sp. antibodies of all major immunoglobulin classes (IgA, IgM, and IgG) are generated against both cell wall and cytoplasmic antigens. Most serological tests for Brucella antibodies employ smooth lipopolysaccharide (S-LPS), the immunodominant cell wall antigen that is shared by all smooth species, *B. abortus*, *B. melitensis*, and *B. suis*. Because *B. canis* is a naturally rough species, a homospecific antigen is required to diagnose infection with this organism.[20,21,38]

Since it was first developed in 1897, serum agglutination has been the mainstay of brucella serodiagnostics.[39] The antigen preparation is standardized and the results can be expressed in international units that can be correlated well with clinical signs of infection. The problem of defining a serum agglutination titer (SAT) indicative of active infection remains a problem; however, most authorities consider $\geq$1:160 (200 IU) to be diagnostic when combined with compatible historical and clinical findings.[40] Qualifications of the SAT include cross-reactions with antibodies to other organisms, including *Francisella tularensis, Vibrio cholerae,* and *Yersinia enterocolitica* serotype O:9, and a prozone phenomenon with high levels of antibody. These problems can be overcome by routinely diluting serum $>$1:320. On rare occasions, the serum of a patient with bacteriologically confirmed brucellosis will fail to agglutinate owing to the presence of a "blocking" substance. So-called blocking antibodies are generally IgG or IgA isotype and can be detected using Coombs test.[41]

The SAT measures the total quantity of *Brucella* agglutinins, but it does not distinguish between IgM and IgG isotypes. One method to do this is based on the action of mercaptans, such as 2-mercaptoethanol (2ME) or dithiothreitol (DTT) to disrupt the disulfide bonds of the IgM pentamer and negate its ability to agglutinate.[42] The SAT, using phosphate-buffered saline (PBS) as the serum diluent, is performed in parallel with the SAT using PBS containing 0.05 M 2ME (or DTT). Any decline in titer that is observed between the two tests represents the content of 2ME-susceptible (IgM) antibodies.[43] The validity of this interpretation has recently been challenged by studies showing that in selected cases, both IgA and IgG can interfere with the action of mercaptans.[44]

Perhaps the most significant advance in *Brucella* serologic tests has been the development of immunoassays, such as the enzyme-linked immunosorbent assay (ELISA).[45,46] A variety of *Brucella* antigens have been studied, including whole cells, S-LPS, outer membrane proteins, and cytoplasmic proteins.[47] The measurement of specific IgA, IgM, and IgG isotypes is the most sensitive method available. A decline in IgG antibodies is associated with a good clinical response to therapy, whereas persistence of such antibodies is associated with chronic infection or relapse.[48]

Among rapid diagnostic tests for brucellosis, rose bengal agglutination is a qualitative plate assay that is useful for screening purposes; however, positive reactions must be confirmed with a more sensitive quantitative method. A new dipstick assay for detecting IgM antibodies to S-LPS is simple to perform, can be applied to field conditions, and shows a good correlation with other methods.[49] Multiple antigen tests, such as the febrile agglutinin screen, should not be used to diagnose brucellosis since they lack specificity and are difficult to interpret.[50]

Since brucellae are facultative intracellular pathogens, cell-mediated immunity appears to be more important in recovery than humoral factors.[51] A variety of tests are available to test cellular immunity, and while useful for understanding the pathogenesis of infection, none are useful for diagnostic purposes. In addition, skin tests are not recommended for diagnostic purposes because there are no standardized preparations, they have the potential to induce a humoral response, and they do not identify patients with active infection.[52]

4. Biological Characteristics of the Organism

Brucellae are small gram-negative coccobacilli measuring about 0.6 μm long by 0.5–0.8 μm wide. They lack a capsule, endospores, flagellae, or native plasmids. The outer cell membrane resembles other gram-negative bacilli with a dominant lipopolysaccharide (LPS) component. Most strains do not have an obligatory requirement for carbohydrates; however, the growth of *B. abortus* strains is enhanced by erythritol, which is used in preference to glucose as an energy source. Metabolism is oxidative and all strains are aerobic, although some require an atmosphere of 5–10% CO_2 for primary isolation.[53] All brucellae are oxidase and catalase

positive. Differentiation between species and biovars is based on biochemical reactions, growth on media containing dyes, agglutination by specific antisera, and susceptibility to lysis by specific bacteriophages (Table 1). Recently, analysis of fragment lengths of DNA cut by various restriction enzymes has been used to differentiate *Brucella* sp.[54]

Brucella spp. are alpha-*Proteobacteria* phylogenetically related to plant pathogens and symbionts such as *Rhizobium* and *Agrobacterium*, intracellular animal parasites such as *Bartonella* and *Rickettsia*, and to opportunistic and free-living bacteria such as *Ochrobactrum* and *Caulobacter*.[55] Originally the genus was classified into six species (*B. melitensis, B. abortus, B. suis, B. neotomae, B. ovis*, and *B. canis*) and subdivided into biovars or biotypes, based primarily on natural hosts. When the results of DNA–DNA hybridization studies showed a high degree of relatedness among *Brucella* sp., it was proposed that the genus be reclassified as a single species (*B. melitensis*) with the other nomen species classified as biovars.[55,56] However, such a single-species organization does not accurately reflect the observed differences in pathogenicity, host preferences, and evolutionary history,[57] and clinicians prefer the nomen species designation for epidemiological considerations.

Most *Brucella* sp. contain two circular chromosomes of approximately 2.1 and 1.15 Mb, except *B. suis* biovar 3 which has a single chromosome of 3.1 Mb.[58] Sequencing of the genomes of *B. suis*,[59] *B. melitensis*,[60] and *B. abortus*[61] confirmed the relatedness of the species and the presence of a small number of unique genes located on chromosomal regions called genetic islands.[62] It has been suggested that the low rate of genetic exchange among intracellular bacteria such as brucella can be attributed to their isolated location within cells of the host. Despite rapid advances in our knowledge of the genetics of brucellae, the factors relating to virulence and host specificity remain elusive.[63]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

In the United States, the incidence of human brucellosis decreased after World War II from more than 6,000 cases per year (4.5 cases per 100,000 population) in 1948 to approximately 100 cases per year (0.05 cases per 100,000 population) in 2006 (Figure 1). This decline can be attributed to efforts to eliminate brucellosis in animals and laws requiring pasteurization of milk for human consumption. The situation worldwide is less encouraging, and infection predominantly caused by *B. melitensis* remains at epidemic levels in some areas, notably the Middle East. For example, in 2005, the Ministry of Health of Iran reported 25,755 cases of human brucellosis for an incidence of 38/100,000 population.[64] To some extent, this represents better awareness of the problem, better surveillance, and case identification.[65]

5.1.1. High-Risk Categories. Traditionally, brucellosis has been an occupational hazard for farmers, ranchers, veterinarians, abattoir workers, and laboratory personnel. In recent years, the epidemiology of brucellosis has changed in some areas. For example, in Texas, between 1977 and 1986, the majority of patients with brucellosis were Caucasian men with documented exposure to cattle or swine in the course of their occupations. In contrast, between 1982 and 1986, the preponderance of cases occurred in Hispanics of both sexes and the source of infection was unpasteurized goat's milk cheeses originating in Mexico.[66] The shift from occupationally acquired brucellosis to foodborne transmission has also been reported in California.[67,68] Tourists visiting countries where brucellosis is enzootic are also at risk especially if they partake of local food "delicacies" such as goat's milk cheese or candy.[69] Moreover, with the speed of international travel, the symptoms of brucellosis may not become apparent until the traveler returns to the United States.[70]

Brucellosis is a recognized hazard for laboratory personnel working with clinical specimens containing brucellae.[35–37] Although infection with any species of *Brucella* can occur from laboratory accidents,[71] in view of its greater virulence, *B. melitensis* accounts for the majority of cases.[72,73] Laboratory personnel with exposure to infectious aerosols may benefit from antibiotic prophylaxis, whereas lesser exposure can be followed with antibody titers.[74] Although brucellosis is not generally considered a nosocomial pathogen, hospital personnel should employ bloodborne pathogen precautions when caring for patients with brucellosis.[75]

5.2. Epidemic Behavior and Contagiousness

Foodborne outbreaks caused by *B. melitensis* have been reported among individuals ingesting unpasteurized goat's milk cheeses.[76] Such outbreaks often involve entire families, including children, without direct contact with animals. In contrast, infection with *B. abortus* is more often sporadic and usually involves direct contact with diseased animals or their parts. Generally, infection caused by *B. suis* has occurred in abattoirs where infected swine are slaughtered.[77–79] In recent years, with the increasing popularity of hunting feral swine, human infection with *B. suis* has been traced to the killing and dressing of these animals in the Southern United States and elsewhere.[80,81]

Table 1. Differentiation of *Brucella* Species Pathogenic for Man[a]

Species and biotypes		CO_2 requirement	H_2S production	Growth on dyes[b]		Agglutination with monospecific sera[c]		Lysis by phage Tb at RTD[d]	Metabolic tests				Common host Reservior
				Thionin	Basic fuchsin	A	M		Glutamic acid	Ornithine	Ribose	Lysine	
B. melitensis	1	−	−	+	+	−	+	−	+	−	−	−	Sheep, goats
	2	−	−	+	+	+	−	−	+	−	−	−	Sheep, goats
	3	−	−	+	+	+	+	−	+	−	−	−	Sheep, goats
B. abortus	1	+	+	−	+	+	−	+	+	−	+	−	Cattle
	2	+	+	−	−	+	−	+	+	−	+	−	Cattle
	3	+	+	+	+	+	−	+	+	−	+	−	Cattle
	4	+	+	−	+	−	+	+	+	−	+	−	Cattle
	5	−	−	+	+	−	+	+	+	−	+	−	Cattle
	6	−	+	+	+	+	−	+	+	−	+	−	Cattle
	9	+	+	+	+	−	+	+	+	−	+	−	Cattle
B. suis	1	−	++	+	−	−	−	−	±	+	+	+	Pigs
	2	−	−	+	−	−	−	−	+	+	+	−	Pigs
	3	−	−	+	+	−	−	−	+	+	+	+	Pigs
	4	−	−	+	−	+	−	−	+	+	+	+	Reindeer, caribou
	5	−	−	+	−	+	−	−	+	+	+	+	Rodents
B. canis	1	−	−	+	−	−	−	−	+	+	+	+	Dogs

[a]Modified from Corbel.[53]
[b]Growth on Albimini or trypticase agar with added dyes: thianin 20 μg/ml; basic fuchsin 20 μg/ml.
[c]A, *B. abortus*; M, *B. melitensis*.
[d]Tbilisi (Tb) phage used at routine test dilution (RTD).

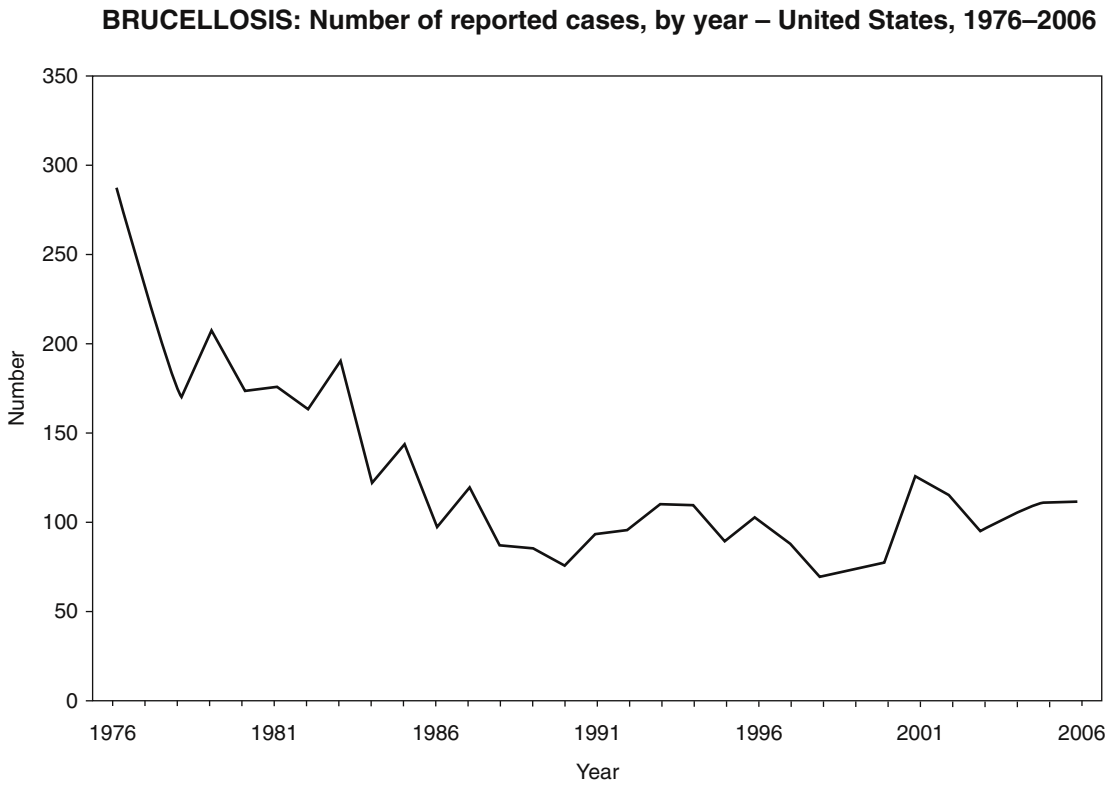


Figure 1. The incidence of brucellosis has remained stable in recent years, reflecting an ongoing risk for infection with *Brucella melitensis* and *B. abortus* acquired through exposure to unpasteurized milk products in countries with endemic brucellosis in sheep, goats, and cattle and *B. suis* acquired through contact with feral swine in the United States.

Brucellosis in humans is not as contagious as it is in animals and human-to-human transmission is rare. Nevertheless, brucellae have been isolated from banked spermatozoa[82] and cases of presumed venereal transmission have been reported.[83,84] A case of *B. melitensis* isolated from human milk also suggests that women with brucellosis should avoid breast-feeding until the disease is treated.[85] Interestingly, unlike other zoonoses, patients with human immunodeficiency virus (HIV) infection do not appear to be at greater risk of brucellosis[86] nor do they have a more severe course when infected.[87]

5.3. Geographic Distribution

Brucellosis occurs worldwide, but is especially prevalent in the Mediterranean basin, the Arabian Peninsula, the Indian subcontinent, and in parts of Mexico, Central and South America. It has been long known that the incidence of human brucellosis is a reflection of the prevalence of the disease in animals. This is illustrated by the concentration of human cases in states that border Mexico where the disease remains enzootic in goats and where goat's milk products cross the border as a traditional food item. Brucellosis in the US region bordering Mexico occurs at a rate eight times higher than elsewhere in the country.[88] The influence of war and economic hardship on the incidence of brucellosis is illustrated by epidemic proportions of the disease in Iran, Iraq, Kuwait, Afghanistan, and states of the former Soviet Union. The situation in sub-Saharan Africa is complicated by a general lack of facilities for diagnosing and reporting cases of brucellosis. A current assessment of worldwide brucellosis has been reported by Pappas and colleagues.[65]

5.4. Temporal Distribution

Although brucellosis can be contracted at any time of the year, seasonal trends have been reported. In a series from California the onset of symptoms occurred between February and May in about one half of bacteriologically confirmed cases.[67] A similar distribution was reported in cases from Texas.[66] In Saudi Arabia, cases were noted to peak in the spring, when many people camp in the desert and exposure

to animals increases.[89] In the Southern Hemisphere where the seasons are reversed, the disease occurs primarily in the fall. Outbreaks of human brucellosis in Peru between 1939 and 1967 coincided with epizootics in goats. Years with abundant rainfall were followed by increased animal parturition, more susceptible goats, and increased milk and cheese production.[90]

5.5. Age and Sex Distribution

Brucellosis can afflict persons of all ages; however, occupation-associated disease reflects the workforce, and men aged between 20 and 60 years have traditionally been at greatest risk.[91] In countries where *B. melitensis* is enzootic, including Mexico and Saudi Arabia, women are more often infected as a result of food exposure.[92] Once considered rare in children, in many areas children less than 15 years of age account for up to 40% of brucellosis cases.[93]

5.6. Brucellosis During Pregnancy

Spontaneous abortion is the principal manifestation of brucellosis in animals and localization of brucellae within the reproductive organs of both sexes is well known. Erythritol is found in the placenta and fetal tissues of pregnant cows and it has been shown to be a growth stimulant for *B. abortus*.[94] Erythritol is not found in human placental tissues and the role of brucellosis as a cause of abortion in humans was questioned.[95] In a retrospective study from Saudi Arabia, Khan and colleagues reported a 46% incidence of spontaneous abortion and intrauterine death among 92 pregnant women with brucellosis.[96] This figure exceeded the rate of spontaneous abortion among the general population during corresponding trimesters. Moreover, the authors found that early diagnosis and treatment prevented abortion and fetal death.

5.7. Innate Immunity

Although there are no known racial or genetic differences in susceptibility to brucellosis in humans, innate immunity has been demonstrated in a variety of animal species. Cameron and associates first demonstrated genetically determined resistance in swine infected with *B. suis* in the 1940s.[97] Using inbred cattle, Templeton and Adams demonstrated high levels of resistance to *B. abortus* mediated by a gene complex termed Nramp-1 (natural resistance-associated macrophage protein 1) located on the bovine chromosome 2.[98] The Nramp-1 gene is expressed primarily in tissue of the RES, and macrophages from resistant cattle showed an enhanced ability to control intracellular replication of brucellae.[99]

6. Mechanisms and Routes of Infection

Brucella abortus is usually transmitted via direct cutaneous inoculation through minor cuts and abrasions in the unprotected skin. Oral transmission via unpasteurized milk can also occur, but *B. abortus* is less pathogenic than *B. melitensis* by this route.[13] Veterinarians immunizing cattle with *B. abortus* strain 19 are at risk from accidental needle sticks or eye splashes with this live attenuated vaccine.[100] The transmission of *B. melitensis* is predominantly via ingestion of unpasteurized milk or milk products from goats, sheep, and, in some areas of the world, camels. Transmission of *B. suis*, other than biovar 4, is generally associated with the slaughter of swine. Outbreaks in abattoirs occur principally in the kill areas where workers are exposed to aerosols of blood that can enter the respiratory tract or through the conjunctivae of the eyes.[77,78] Hunters of infected feral swine are at risk of direct inoculation of *B. suis* during the act of dressing the kill unless they take precautions to prevent exposure to blood.

6.1. Brucellosis in Wildlife

Although brucellosis occurs mainly in domestic and semi-domestic animals, a variety of wildlife are susceptible including elk, caribou, and bison. The role of wildlife as a source of brucellosis for domestic animals continues to be debated despite evidence that under experimental conditions, bison-to-cattle transmission of *B. abortus* was demonstrated. Similarly the role of brucellae isolated from marine mammal and their potential to cause human disease remains to be fully elucidated.[101]

7. Pathogenesis and Immunity

The incubation period of human brucellosis is variable depending in part on the *Brucella* spp., the route of transmission, the size of the inoculum, and the immune and nutritional status of the host. The majority of patients become symptomatic within 1–4 weeks after infection and the onset is insidious in about one half of the cases. Infection caused by *B. melitensis* or *B. suis* tends to be more severe than *B. abortus* or *B. canis*.[68] Although morbidity is considerable, death occurs in less than 1% of cases.

Normal human serum has limited bactericidal activity against *Brucella* spp. and is greatest against *B. abortus*, moderate against *B. melitensis* and *B. suis*, and undetectable against *B. canis*.[102] Chemotactic factors attract neutrophils

to the site of infection where brucellae opsonized by complement are phagocytized. In vitro studies using neutrophils from normal subjects indicated that *B. abortus* is killed more efficiently than *B. melitensis*.[103] The mechanisms by which brucellae survive within neutrophils are poorly understood; however, virulent strains of *B. abortus* appear to contain a potent superoxide dismutase enzyme and nucleotide substances (adenine and guanosine monophosphate) that inhibit phagolysosome fusion, degranulation, and activation of the myeloperoxidase–halide system.[104] Smooth lipopolysaccharide (S-LPS) appears to play an important role in intracellular survival as smooth organisms survive more efficiently than non-smooth organisms.[105] Organisms that escape killing by neutrophils enter the lymphatics where they localize within organs of the RES. Survival within macrophages is dependent upon specific proteins including stress proteins, such as HtrA, that is also involved in the granulomatous response.[106] Replication of brucellae continues within macrophages until these cells are "activated" by specifically committed T-lymphocytes. CD-4 cells play a role either by activating CD-8 T-cells or by secreting cytokines, especially interferon-γ.[107] Although humoral antibodies appear to play some role in resistance to brucellosis, cell-mediated immunity predominates.[108]

8. Patterns of Host Response

The host response in brucellosis is somewhat dependent upon the *Brucella* spp. and the tissue in which it is viewed. Characteristically, infection with *B. abortus* induces a granulomatous reaction which can be demonstrated in the bone marrow, liver, spleen, and lymph nodes. Granulomas are detected in bone marrow in up to 75% of cases; however, they are often small and have indistinct borders.[109] Occasionally there is an increase in histiocytes showing erythrophagocytosis that may correlate with severe thrombocytopenia.[110]

The liver is the best studied organ and percutaneous liver biopsies from patients infected with *B. abortus* characteristically reveal noncaseating epithelioid granulomas indistinguishable from sarcoidosis.[111] In contrast, infection with *B. melitensis* can produce a spectrum of histologic features including small aggregates of mononuclear cells around necrotic foci, a diffuse mononuclear cell infiltrate resembling viral hepatitis, and occasionally loose granulomas containing histiocytes.[112] Infection caused by *B. suis* characteristically produces suppurative abscesses that can become chronic and can reactivate many years later.[113] Localized infection with either *B. suis* or *B. melitensis* can give rise to so-called hepatosplenic brucellosis.[114,115]

8.1. Clinical Features

The spectrum of human brucellosis ranges from subclinical to chronic. Whether the onset is acute or insidious, it is characterized by a myriad of nonspecific complaints including fever, sweats, weakness, fatigue, headache, arthralgia, and depression. When untreated the fever pattern is undulating, but follows the usual diurnal variations. In contrast, there is often a paucity of abnormal physical findings, notably fever and hepatosplenomegaly. Treatment with appropriate antimicrobial drugs shortens the course of illness and lessens complications. Any organ or system of the body can be involved, but osteoarticular complications are the most frequent occurring in up to 60% of cases in some series.[116] A variety of bone and joint lesions have been reported, but sacroiliitis is reportedly the most common.[117,118]

Other complications of brucellosis can involve respiratory, gastrointestinal, hepatobiliary, genitourinary, cardiovascular, and nervous systems. With treatment most patients recover fully within weeks to months; however, as many as 15% relapse depending on the antimicrobial agents used and the length of therapy. Clinical relapse generally occurs within 3–6 months after stopping therapy and is usually not caused by the emergence of antibiotic-resistant strains.[119] The causes of relapse are multifactorial, but discontinuation of antibiotics before completing a full 6-week course is an important consideration. The majority of patients who relapse can be treated successfully by repeating a full course of the same antibiotics. Rarely chronic infection occurs, during which patients can experience bouts of fever and other symptoms and the titer of IgG antibodies remains elevated. The cause is usually a focus of infection in the tissues that may require surgical intervention and prolonged antibiotics. A minority of patients experience a delayed convalescence during which they complain of prolonged illness, but they can be distinguished from chronic brucellosis by the absence of fever and antibody titers are not elevated.[120]

8.2. Diagnosis

The diagnosis of brucellosis requires a high index of suspicion derived from a history that includes occupation, avocations, exposure to animals, ingestion of high-risk foods, and travel to areas where the disease is prevalent. Routine laboratory tests are generally not helpful, except that the WBC count is often normal or low.[121] Although patients develop cutaneous hypersensitivity to brucella antigens, skin tests are not useful in making the diagnosis. A definitive diagnosis is made by recovering brucellae from blood, bone marrow, or other tissues. In the absence of bacteriologic

confirmation, the diagnosis rests with the demonstration of high or rising titers of specific antibodies in the serum.[40]

9. Control and Prevention

9.1. General Concepts

Despite the availability of effective vaccines to prevent brucellosis in animals, the disease remains enzootic in many countries. What then hampers the control and eradication of this important and widespread zoonosis? In 1993, the International Task Force for Disease Eradication concluded that brucellosis would not be targeted for eradication worldwide because of a "low political will".[122] In part, this reflects the high cost of brucellosis eradication programs, but it also indicates the failure of governments to recognize the public health implications of animal diseases. Epidemiologic studies have shown that the persistence of brucellosis in animals and man's risk of contracting the disease are closely linked to (a) methods of animal husbandry, (b) standards of hygiene, and (c) habits and customs associated with the preparation of foods. Brucellosis in humans can be prevented by attention to these issues, most notably the prevention of brucellosis in animals through immunization.[123]

9.2. Chemotherapy and Prophylaxis

A variety of antimicrobial agents are effective for treating brucellosis, but in vitro susceptibility does not always predict clinical effectiveness.[124] The most effective therapy for human brucellosis is doxycycline (100 mg twice daily by mouth for 6 weeks) plus either streptomycin (1 g intramuscular for 2–3 weeks) or gentamicin (5 mg/kg/day intramuscularly for 1 week).[125] An alternative and completely oral regimen is doxycycline (100 mg twice daily by mouth) plus rifampin (600–900 mg daily by mouth) both given for 45 days. Although this regimen gives comparable results for uncomplicated illness, the relapse rates are higher in patients with osteoarticular complications.[126] For the treatment of brucellosis in children less than 8 years of age and pregnant women, for whom tetracycline is contraindicated, trimethoprim–sulfamethoxazole (TMP/SMZ; four tablets each containing 80 mg TMP/400 mg SMZ) daily by mouth for a minimum of 21 days has been recommended. The optimal treatment of brucella endocarditis and neurobrucellosis has not been determined. Most authorities recommend combination of drugs, including doxycycline, rifampin, and TMP/SMZ administered for prolonged periods of time. Some quinolones have good in vitro activity, but are not first-line therapy even when combined with other drugs.[127]

9.3. Immunization

Vaccines for use in animals were used at one time to immunize humans at high risk in the Soviet Union and China. Such vaccines are not approved for human use in the United States as they are live vaccines and have the potential to cause infection. Inactivated brucella vaccines and various antigenic preparations have been studied in humans, but to date they have not proven effective.

10. Unresolved Problems

With the importance of *Brucella* spp. as potential biowarfare agents, there is a need for a vaccine to protect humans. Because live vaccine strains have the potential for producing illness, there is interest in subunit vaccines that are not pathogenic, but will induce long-lasting immunity. To this end, there is a need to determine virulence antigens against which subunit or DNA vaccines can be directed.

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CHAPTER 9

Campylobacter Infections

Ban Mishu Allos

1. Introduction

Campylobacters are slender, spiral or curved, microaerophilic gram-negative rods that are one of the most commonly reported bacterial causes of diarrhea in the developing and developed world. Campylobacters also may cause systemic illness in humans and a number of diseases in wild and domestic animals. Because of morphological similarity with vibrios, these organisms were originally classified as *Vibrio fetus*.[1] *Campylobacter* (Greek for "curved rod") was proposed as a name of a new genus when it was found that these organisms differed in their biochemical characteristics from true members of the genus *Vibrio. C. jejuni* is now regarded as among the leading causes of diarrheal disease in humans. The complete genomic sequence of *C. jejuni* was characterized in 2000.[2] This may open the door for new insights into the behavior of these organisms. Worldwide, more than 80% of *Campylobacter* isolates implicated in human disease are *C. jejuni*; however, culture media commonly used to identify campylobacters may not support other potentially pathogenic *Campylobacter* species. Other *Campylobacter* or *Campylobacter*-like species that have been associated with human disease include *C. coli, C. fetus*, subsp.*fetus, C. lari, Helicobacter fennelliae, H. cinaedi, C. hyointestinalis, C. upsaliensis, C. jejuni* subsp. *doyleii, C. sputorum*, and*Arcobacter butzleri* (Table 1). *C. fetus* subsp. *fetus* also causes systemic infections in immunocompromised hosts. Some campylobacters and related species such as *C. nitrofrigilis*, *A. cryaerophila*, and *C. concisus* have not yet been associated with human disease. *Helicobacter pylori* is associated with gastritis, peptic ulcer disease, and gastric cancer in humans. *H. pylori* will not be discussed in this chapter.

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2. Historical Background

The first identification of *Campylobacter* was in 1909, during an investigation of infectious abortion, when MacFadyean and Stockman,[3] two English veterinarians, isolated organisms from placentas and aborted fetuses of cattle that they called *Vibrio fetus*. Subsequently, similar organisms were found to cause infectious abortion in sheep.[3] *V. fetus* became known as a major cause of enzootic and epizootic abortion in cattle and sheep.[4] Over the next 30 years, these organisms were isolated from calves[5] and swine with diarrhea[6] and from fowl with hepatitis and diarrhea.[4] Although *V. fetus* was at first believed to cause diarrheal illness in these hosts, this association has not been clearly documented.

The first isolation of *V. fetus* from a human was in 1947 from the bloodstream of a pregnant woman who had an infectious abortion.[7] Over the next 30 years, there were occasional reports in the medical literature of isolation of these organisms from the bloodstream, cerebrospinal fluid, joint aspirates, endovascular tissue, and abscess cavities. Most of the hosts were debilitated by alcoholism, neoplasm, diabetes mellitus, cardiovascular disease, or old age.[8,9] *V. fetus* was thought to be an unusual cause of systemic illness in debilitated hosts, and it was thus considered to be an opportunistic organism. However, in 1957, Elizabeth King[10] at the Communicable Disease Center (CDC) recognized that not all the *V. fetus* organisms were the same; she identified two groups with distinct serological and biochemical characteristics. The majority of the isolates grew at 25 and 37°C, but the rest grew only at 37 and 42°C. She called this latter group "related vibrios" and noted that although they were isolated from the bloodstream; in each case the patient had had a diarrheal illness. She postulated that the related vibrios caused acute diarrheal illness, but because they were slow growing and fastidious, they could not be isolated from fecal specimens.[10]

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Table 1. Biochemical Characteristics of *Campylobacter* and Related Species[a]

Organism	Cat[b]	Nit red[c]	Ind ace[d]	Aryl sulf[e]	Pyrazin[f]	Hipp[g]	Nal[h]	Ceph[i]	H_2S Rapid[j]	H_2S Lead ace[k]	TSI[l]	Growth at 25°C	Growth at 37°C	Growth at 42°C	H_2 required
C. jejuni bio 1[m]	+	+	+	−	+	+	S	R	−	++	−	−	+	+	−
C. jejuni bio 2	+	+	+	+	+	+	S	R	+	++	−	−	+	+	−
C. coli	+	+	+	−	+	−	S	R	−	++	−	−	+	+	−
C. fetus	+	+	−	−	−	−	R	S	−	+	−	+	+	−	−
C. upsaliensis	(+)	+	+	−	+	−	S	S	−	(+)	−	−	+	(+)	[n]
C. lari	+	+	−	−	+	−	R	R	+	+	−	−	+	+	−
C. hyointestinalis	+	+	−	−	−	−	R	S	−	5+	3+	(+)	+	+	[n]
H. fennelliae[o,p]	+	−	+	+	−	−	S	(S)	−	+	−	−	+	−	−
H. cinaedi[o]	+	+	−	−	−	−	S	(S)	−	(+)	−	−	+	−	−
C. jejuni s. *doylei*	(+)	−	+	−	+	(+)	S	(S)	−	−	−	−	+	(+)	[n]
A. cryaerophilis[q]	+	+	+	−	−	−	S	(R)	−	−	−	+	+	−	−
A. butzleri[q]	(+)	+	+	−	−	−	S	(R)	−	−	−	−	+	(+)	−
C. sputorum bio. *sputorum*	−	+	−	+	+	−	R	S	+	5+	3+	−	+	+	−
C. sputorum bio. *bubulis*	−	+	−	+	−	−	R	S	+	5+	3+	−	+	(+)	−
C. sputorum bio. *faecalis*	+	+	−	+	+	−	R	S	+	5+	3+	−	+	+	−
C. concisus	−	+	−	+	+	−	(R)	S	−	3+	(+)	−	+	(−)	+
C. mucosalis	−	+	−	−	−	−	R	S	−	5+	+	(−)	+	(−)	+
C. curvus	−	+	+	+	+	−	R	S	−	5+	+	−	+	+	+
C. rectus	−	+	+	+	+	−	S	R	−	3+	+	−	+	+	+

[a] +, Positive; (+), most strains positive; −, negative; (−), most strains negative; R, resistant; (R), most strains resistant; S, susceptible; (S), most strains susceptible.
[b] Catalase.
[c] Nitrate reduction.
[d] Indole acetate.
[e] Aryl sulfatase.
[f] Pyrazinamidase.
[g] Hippurate hydrolysis.
[h] Nalidixic acid resistance.
[i] Cephalothin resistance.
[j] Rapid H_2S. Method of Skirrow and Benjamin.(249)
[k] Lead acetate.
[l] Triple sugar iron.
[m] *C. jejuni* subsp. *jejuni* biotypes 1 and 2 refer to Skirrow's scheme. Susceptibilities are based on 30 μg disks.
[n] Some isolates grow much better in H_2-enhanced growth conditions.
[o] Spreading, noncolonial growth.
[p] Hypochlorite odor.
[q] Aerobic growth occurs at 30°C.

Based on King's and his own observations that the related vibrio was associated with diarrhea, Bokkenheuser[8] concluded that this organism was probably present in feces, but could not be detected with the available laboratory techniques. Cooper and Slee[11] in Australia isolated *V. fetus* (*C. jejuni*) from the stool of an immunocompromised patient with recurrent bacteremia and diarrhea by inoculating the patient's feces onto a horse blood agar plate containing cephalothin disks and incubating it overnight in a carbon dioxide-enriched atmosphere. In 1972, Dekeyser et al.[12] reported a positive stool culture for *Campylobacter* from a 22-year-old woman from whom *C. jejuni* had been isolated from the blood. The stool isolate was obtained by filtering fecal specimens through a 0.65 μm Millipore filter with a syringe. Using this filtration procedure, Butzler et al.[13] were able to isolate *Campylobacter* from 5.1% of 800 children in Brussels with diarrhea and only 1.3% of 1,000 children without diarrhea. Skirrow[14] showed that an agar medium containing antibiotics could be used instead of filtration as a primary isolation technique and that *C. jejuni* was as important a cause of diarrheal disease in England as Butzler et al.[13] had found it in Belgium. The use of antibiotic-containing selective media combined with simple methods for obtaining a microaerobic atmosphere have made isolation of *Campylobacter* a routine procedure and have opened up the study of *Campylobacter* infections on a worldwide scale. In 1973, Veron and Chatlain[15] reported that *V. fetus* and related vibrios had biochemical characteristics dissimilar

to those of the Vibrionaceae, and a new genus, *Campylobacter*, was proposed. Under this scheme and later modification by Smibert,[4] King's related vibrios became *C. jejuni* and *C. coli*, and the opportunistic organisms became *C. fetus* subsp. *fetus*, respectively.[1,15]

New members of the genus *Campylobacter* and related genera are being identified with regularity. Some Campylobacters, particularly the hippurate-negative group, are not thermotolerant and are susceptible to antibiotics, such as cephalothin and colistin, that are incorporated into selective isolation media. These *Campylobacter* can be isolated at 37°C on media without antibiotics by inoculating a stool suspension directly on a 0.45 μm Millipore filter that has been placed directly on a blood agar plate.[16] Using these and other similar methods, increasing numbers of these organisms have been isolated from humans with gastrointestinal illness, suggesting that "atypical" campylobacters play a greater role in causing human disease than previously thought.[17] Nevertheless, at present, *C. jejuni* remains the most important cause of diarrheal disease in this group, and the importance of these "related" campylobacters as human pathogens has not yet been fully defined.

3. Methodology

3.1. Sources of Mortality Data

Limited information is available concerning the importance of *Campylobacter* in fatal diarrheal disease; however, judging from the frequency of *Campylobacter* infection in patients ill enough to seek medical attention, the bacterium may be a major cause of mortality due to diarrheal disease. In developed countries in recent years, mortality due to diarrheal diseases has been low. Several deaths due to infection by *C. jejuni* have been reported, which have allowed for limited population-based estimates of mortality.[18] The uncommon human infections with *C. fetus* subsp. *fetus*, usually occurring in debilitated hosts, have sometimes been fatal.[8] Campylobacter infections occurring in persons with human immunodeficiency virus (HIV) infection or other immunodeficiencies may be associated with higher mortality rates. Guillain–Barré syndrome and the hemolytic–uremic syndrome are uncommon complications of *C. jejuni* infections that are also potentially fatal (Section 8.1).

3.2. Sources of Morbidity Data

Because campylobacters produce diarrheal illnesses that are usually indistinguishable from those caused by other viral and bacterial enteric pathogens, it is not possible to obtain data on morbidity in the absence of laboratory identification of the organism. Most data on morbidity in Europe and the United States come from hospital-based or community-based studies of *Campylobacter* infections.[19–21] Estimates of incidence in HIV-infected persons are made by studying patients in AIDS clinics.[22,23] Surveys from hospitals and clinics have also been conducted in developing countries. At the International Center for Diarrheal Disease Research in Bangladesh, surveillance is based on culturing 4% of patients who come to the center with acute diarrheal disease.[24] In England and Wales, 200 public health and hospital laboratories report *Campylobacter* isolations to the Communicable Disease Surveillance Center.[25] In the United States, *Campylobacter* isolations are routinely reported to the Centers for Disease Control and Prevention (CDC).[26] Outbreaks of *Campylobacter* infection are routinely reported to the CDC *Campylobacter* National Surveillance system.[26]

3.3. Serological Surveys

Serological surveys using one or several antigens common to the majority of *Campylobacter* strains have been performed among healthy populations in the United States, Bangladesh, and Thailand.[27–29] Surveys have been made to confirm the diagnosis in patients with diarrhea, to investigate outbreaks, to determine the etiology when multiple pathogens were isolated, to investigate occupational exposure and other high-risk groups,[30,31] and to investigate the association of *Campylobacter* with postinfection complications such as reactive arthritis and Guillain–Barré syndrome.[31,32] The Foodborne Diseases Active Surveillance Network (FoodNet) of the CDC's Emerging Infections Program collects data from 10 US states regarding diseases caused by enteric pathogens including Campylobacter.[32]

3.4. Laboratory Diagnosis

Stools or rectal swabs, the usual specimens submitted to the laboratory for examination, are inoculated onto selective media to permit isolation of *Campylobacter*. A transport medium such as Cary–Blair should be used if a delay of more than 2 h is anticipated before arrival of the specimen to the laboratory.[33] The three most commonly used selective media for isolation of *C. jejuni* or *C. coli* are Skirrow's,[14] Butzler's,[34] and Campy–BAP,[35] all with rich basal media enriched with sheep or horse blood and all containing antibiotics to inhibit the normal enteric flora. Skirrow's medium is the least inhibitory to the enteric flora; however, in most patients with *Campylobacter* enteritis, the titer of

Campylobacter organisms per gram of stool ranges from 10^6 to 10^9,[36] a quantity easily detected with this medium. Butzler's media, Virion, which contains cefaperazone and is more inhibitory to competing flora, also appears to be very useful.[37] Blood-free media containing antibiotics have also been used successfully,[38] and several enrichment media have been found to support the growth of *Campylobacter.*[37] Enrichment methods are probably not necessary for detection of *Campylobacter* is diarrheal stools, but may be useful in detecting carriers. A combination of blood-free media such as cefoperazone deoxycholate agar and charcoalbased selective medium may achieve the highest yield of *C. jejuni* or *C. coli* from stool specimens.[39]

Many campylobacters that cause diarrhea are inhibited by cephalothin or colistin, which are present in selective media, or by incubation at 42°C.[16,40] In the United States, more than 99% of reported *Campylobacter* isolates are *C. jejuni.*[26] This predominance probably reflects the types of culture media routinely used for the detection of campylobacters in this country. In studies where antibiotic-free media or filtration methods are used, the proportion of campylobacters that are non-*jejuni* species is over 20%. Both Campy–BAP and Butzler's media contain cephalosporins, which inhibit most strains of *C. fetus* subsp *fetus* and many of the "atypical" organisms; for this reason, the potential of these organisms to cause human disease may be underappreciated.[41,42] Steele demonstrated a 0.45 μm cellulose acetate membrane filter placed directly on an antibiotic-free blood agar plate could be used to isolate *Campylobacter* at 37°C.[16] *Campylobacter* in saline solution is able to pass through the filter while other fecal flora do not. A 0.65 μm filter may offer better isolation rates than the 0.45 μm, although the number of contaminating bacteria is also increased. Because the yield for detecting atypical campylobacters is so much higher with a filter and nonselective medium such as chocolate agar,[17] this technique is now recommended for primary isolation of campylobacters from feces or rectal swabs.

Campylobacters require an atmosphere with an oxygen concentration of 5–10% for optimal growth. A microaerobic atmosphere may be produced by using *Campylobacter*-specific gas-generating kits[43] or by evacuating vacuum jars or bags and refilling with a mixture of 5% oxygen, 10% carbon dioxide, and 85% nitrogen. Campylobacters are capnophilic, although carbon dioxide is not essential for growth incubation of plates in a candle jar may permit isolation from most positive specimens, but should best be used at 42°C.

When using selective antibiotic media, optimal isolation of *C. jejuni* is obtained at an incubation temperature of 42°C; however, some *Campylobacter* species cannot be isolated at that temperature (Table 1). All organisms will grow at 37°C. When using the more selective media, the higher temperature for incubation is recommended. If the membrane filter method is employed, the optimum incubation temperature is 37°C.

After incubation of cultures under these conditions for 24–48 hr, colonies that are gray, mucoid, or wetappearing, or discrete should be suspected of being *Campylobacter* and may be identified as such using standard biochemical tests (Table 1). A rapid presumptive identification can be made by the following characteristics: (1) typical colonial morphology, (2) typical Gram's stain showing gram-negative curved rods, (3) indication of oxidase and catalase positivity, and (4) motility. *Campylobacter* can be isolated from body fluids or tissues that do not have a normal flora by using thioglycollate broth or biphasic media. Some commercial blood culture systems are superior to others for detection of bacteremia due to *Campylobacter* species.

Direct examination of stools has been used as a rapid screening test for *Campylobacter* in developed countries.[44–46] These methods are usually specific, but are only about 50–75% sensitive. Staining with 1% aqueous basic fuchsin was reported to be the most sensitive method.[45] Sensitivity was increased in the presence of fecal leukocytes.[46] Serological diagnosis is possible in which antibodies to the homologous strain or to a common acid-extracted antigen are detected.[27]

A biotyping scheme for *C. jejuni, C. coli*, and *C. laridis* strains has been developed that is based on hippurate hydrolysis, rapid H_2S production, and DNA hydrolysis.[47] A phage-typing system has also been developed for *C. jejuni* and *C. coli.*[48] The two most widely used serotyping systems for *C. jejuni* and *C. coli* are the Penner system, which identifies soluble, heat-stable (somatic, O) antigens by passive hemagglutination,[49] and the Lior system, which measures heat-labile (flagellar, H) antigens by slide agglutination.[50] There are over 90 reference serotypes defined by the Penner system and 112 by the Lior system. Both systems identify over 90% of *Campylobacter* isolated from humans and nonhuman sources.[51,52] The Lior system is easier to use and more rapid and consequently more applicable for use outside reference laboratories.[53] Strains of a single serotype in one system may belong to multiple serotypes in another system. These two systems may eventually be combined to produce a system similar to *Escherichia coli* serotyping based on both O and H antigen grouping. Polymerase chain reaction (PCR)-based techniques for identification and typing of Campylobacter species also are now available.[54–56]

4. Proof of Causation and Biological Characteristics of the Organism that Affect the Epidemiological Pattern

The evidence that *C. jejuni* is an etiologic agent for human diarrheal disease can be summarized as follows: (1) In developed countries, *Campylobacter* has been isolated from the stools of 3–14% of patients presenting at hospitals with diarrhea, but only rarely from stool samples of controls (Table 2). (2) Outbreaks of gastrointestinal illness have occurred among previously healthy populations in which no other pathogen has been found, and *Campylobacter* has been isolated from the stools of patients but not from controls (Section 6). (3) In natural settings and in experimental models, *Campylobacter* has caused diarrheal illness in avian species[57] and in mammals such as puppies[58] and nonhuman primates.[59] (4) In several patients who had diarrheal illness, *Campylobacter* was simultaneously isolated from stool and blood.[10–12] (5) Rising titers of specific serum antibody to the infecting organisms have been shown.[27,35] (6) Treatment of ill *Campylobacter*-infected patients with erythromycin, an agent to which most other recognized enteric pathogens do not respond, has been followed by rapid clearance of organisms and rapid remission of symptoms.[60] (7) Two volunteers ingested organisms in a glass of milk and developed a typical gastrointestinal illness after 3 days, and *Campylobacter* was isolated from their stools.[61] (8) Finally, two strains of *C. jejuni* isolated from persons with diarrhea were fed to 111 American adult volunteers with sodium bicarbonate.[62] The minimum infective dose was 1,000 organisms. With one strain (81–176), all became infected (i.e., *Campylobacter* was isolated from their stools) and 18 (46%) of 39 developed diarrhea with fecal leukocytes or fever after ingestion of 10^6 organisms. The findings thus fulfill the Henle–Koch postulates of causation (see Chapter 1, Section 13).

Campylobacters grow best at the body temperatures of warm-blooded hosts. *C. jejuni* has been identified as a part of the enteric flora in swine, dogs, cats, nonhuman primates, rodents, cattle, sheep, and other ruminants.[4]

Table 2. Isolation of *Campylobacter* from Fecal Cultures from Patients with Diarrhea and from Healthy Controls in the Developed Countries, 1973–1986

			Patients with diarrhea				Healthy controls	
Reference	Location	Population	No. studied	% with *C. jejuni*	% with *Salmonella*	% with *Shigella*	No. studied	% with *C. jejuni*
Butzler[13]	Belgium	Children	800	5.1			1000	1.3
Skirrow[14]	England	All ages	803	7.1			194	0
Brunton[266]	Scotland	All ages	196	8.7	2.5	6.7	50	0
Bruce[267]	England	All ages	280	13.9	4.3	3.9	156	0.6
Kendall[20]	England	All ages	3,250	14.9	2.1	1.1		
Severin[268]	Netherlands	All ages	584	10.8	10.0		120	0
Lopez-Brea[269]	Spain	All ages	446	4.5	12.1	1.3		
Delorme[270]	France	All ages	100	9.0	0	1.0	330	0
Graf[271]	Switzerland	All ages	665	5.7	12.6	0.9	800	0
Velasco[272]	Spain	All ages	6,970	7.3	10.2	5.4		
Figura[273]	Italy	Children	643	9.0	9.6	0		
Cevenini[274]	Italy	Children	561	8.0	7.3	0		
Svedhem[21]	Sweden	All ages	2,550	10.9	7.2	3.5		
Walder[210]	Sweden	All ages	5,771	6.9	4.1	1.7	2000	0.25
Lassen[78]	Norway	All ages	7,700	3.0	4.7	2.9		
Steingrimsson[82]	Iceland	All ages	4,019	1.7	4.1	0.2		
Blaser[208]	United States	All ages	2,670	4.6	3.4	2.9	157	0
Blaser[19]	United States	All ages	8,097	4.6	2.3	1.0		
Pien[275]	Hawaii	All ages	471	8.7	4.2	3.8		
Pai[276]	Canada	Children	1,004	4.3	5.1	1.4	176	0
Thompson[81]	Canada	All ages	146,842	3.8	7.9	0.2		
Burke[277]	Australia	Children	975	7.4	5.7	1.3	975	0.6
Kirubakaran[278]	Australia	Children	386	4.4	12.2	3.4	332	0
Skirrow[279]	England	All ages	1,873	5.5	3.4	0.8		

Optimal growth occurs at 42°C, the body temperature of fowl, and *C. jejuni* appears to be commensal in adult domestic fowl. *Campylobacter* appears to be an enteric pathogen for primates,[59] but its role as an enteric pathogen in other animal species is not well defined.

Viability of organisms under environmental conditions is temperature dependent. *C. jejuni* will survive for weeks in water, feces, urine, and milk when kept at 4°C; however, at 25°C, viability persists for a few days or less.[36] *Campylobacter*, like *Salmonella*, is quite sensitive to low pH and will not survive longer than 5 min at a pH less than 2.3. At neutrality or alkaline pH, especially in bile, organisms may multiply and survive up to 3 months at 37°C.[36]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

C. jejuni is an important cause of diarrheal illness in the developed and developing world and in all age groups. Prevalence of *disease* caused by *Campylobacter* is based on the rate of isolation of the organism from patients with diarrheal illness. Inferences drawn from such studies must be tempered by knowledge of the data artifacts implicit in laboratory-based culture surveys. Defining the presence of *infection* due to *Campylobacter* in a community includes prevalence of isolation of the bacterium from the stools of healthy persons and the presence of antibodies to *Campylobacter* among a representative population sample.

Campylobacter infections have been evaluated as part of surveys on the etiology of diarrheal disease among persons coming to the hospital or clinic. In the developed world, *Campylobacter* ranks among the most commonly isolated bacterial pathogens from persons with diarrheal disease and is uncommonly isolated from populations of healthy controls (Table 2). Studies in communities, cities, and states in the United States, England, and Israel show that the rate of reported *Campylobacter* isolations ranges from 1.4 to 100 per 100,000 population per year.[18,25,63,64] Rates of *Campylobacter* infections vary according to the age distribution and location of the study population. The rate of *Campylobacter* isolation in the United States reported to the CDC has been stable at 5–6 per 100,000 population since 1982.[26] Because not all hospital laboratories routinely culture diarrheal stools, this reported rate likely represents an underestimate of the true rate of *Campylobacter* infections.

Two prospective studies document the true incidence of campylobacters relative to salmonellae and shigellae. In the first, eight hospital microbiology laboratories routinely looked for campylobacters every time they cultured stools for other pathogens. *Campylobacter* was isolated twice as frequently as *Salmonella* and 4.5 times as frequently as *Shigella.*[19] In the second study, a Seattle health maintenance organization (HMO) cultured all diarrheal stools for the same three pathogens. Campylobacters were again isolated twice as frequently as salmonellae and seven times as frequently as shigellae.[65] Because the reported rate of *Salmonella* infections is 20 per 100,000 population, if clinical laboratories cultured diarrheal stools as often as they cultured them for salmonellae, the incidence of *Campylobacter* infections would be twice this rate—40 per 100,000 population. Similarly, in a 1999 CDC study, date were collected on foodborne infections from numerous sources including several surveillance systems, surveys, and other published population-based studies.[66] They estimated a total of 2.4 million cases of *Campylobacter* infection occurred in the United States each year—more than the estimated number of cases of *Salmonella* (1.4 million) and *Shigella* (448,240) combined.

Campylobacter infections may be more underreported than other bacterial enteric infections because of the mildness of many infections; patients might not find it necessary to seek medical care. In a community-wide outbreak of *C. jejuni* infections caused by a contaminated water supply, only 1 of 18 symptomatic persons sought medical care.[67] In another outbreak at a summer camp, only 4 (10%) of 41 infected persons were sufficiently ill to seek medical attention.[68] These and other studies indicate that the true annual incidence of *Campylobacter* infections in the United States is 1,000 per 100,000 population.[26] Similarly, in England the reported rate of *Campylobacter* infections is only 20 per 100,000 population per year; however, studies at the level of community-based physicians also showed that the true annual incidence is 1,100 per 100,000 population.[20] The case-fatality rate for *Campylobacter* infections has been estimated at 0.05 per 1,000 cases.[18]

Most recently, surveillance for *Campylobacter* infections has shown a downward trend in rates of infection. According to the active population-based surveillance network, FoodNet established by CDC and other federal agencies, the incidence of *C. jejuni* infections per 100,000 population in the United States fell by more than 30% from 21.7 in 1998 to 12.9 in 2003.[32]

The epidemiology of *Campylobacter* infections is markedly different in tropical developing countries. In such countries, *Campylobacter* infection is hyperendemic and asymptomatic infection may be frequent (Table 3). In countries such as Bangladesh, South Africa, The Gambia, Zaire, India, Australia, Indonesia, and China, the rate of *Campylobacter* infections is greatest in infants and declines with age.[69] Estimates from laboratory-based surveillance suggest the incidence of this infection among children less

Table 3. Isolation of *Campylobacter* from Fecal Cultures from Patients with Diarrhea and from Healthy Controls in the Developing Countries

				Patients with diarrhea		Healthy controls	
Reference	Location	Year	Population	No. studied	% with *Campylobacter*	No. studied	% with *Campylobacter*
	Asia						
Ringertz[280]	Indonesia	1980	0–9 yr	144	10	7	29
Blaser[86]	Bangladesh	1980	Children	204	12	141	18
Rajan[281]	India	1982	All ages	305	15	–	–
Glass[72]	Bangladesh	1983	All ages	3038	14	333	7
Nair[282]	India	1983	All ages	116	10	–	–
Lim[283]	Malaysia	1984	All ages	–	4	–	2
Ho[192]	Hong Kong	1985	0–2 yr	1957	2	1841	2
Young[284]	China	1986	0–15 yr	48	19	105	9
Taylor[a] [123]	Thailand	1986	0–5 yr	1230	13	1230	11
Nath[285]	India	1993	Children	604	4	529	1
	Europe						
Popovic-Urovic[286]	Yugoslavia	1989	All ages	2080	16	–	–
	Africa						
De Mol[83]	Rwanda	1983	Children	271	14	203	3
Olusanya[287]	Nigeria	1983	5–39 yr	–	12	–	2
Georges[288]	Central African Republic	1984	0–15 yr	1197	11	748	7
Pazzaglia[289]	Egypt	1993	Children	880	17	1079	6
Zaki[290]	Egypt	1986	All ages	3135	2	702	1
Bokkenheuser[8]	South Africa	1979	0–2 yr	78	35	63	16
Mauff[291]	South Africa	1981	All ages	2323	4	–	–
	South Africa	1981	0–2 white	–	5	–	–
	South Africa	1981	0–2 black	–	13	–	–
Mackenjee[292]	South Africa	1984	Hospital	126	21	128	5
	South Africa	1984	Outpatient	352	7	–	–
Lastovica[293]	South Africa	1986	Children	2951	10	–	–
	Latin America						
Hull[294]	Trinidad	1982	Children	60	12	–	2
Mata[295]	Costa Rica	1983	Children	233	8	–	–
Guerrant[296]	Brazil	1983	Children	40	8	–	–
Olarte[297]	Mexico	1983	Children	265	9	54	4
Figueroa[298]	Chile	1986	Children	35	6	–	–
Asrat [299]	Ethiopia	1993	0 to 5 years	630	14	220	1
Koulla-Shiro[300]	Cameroon	1990	0 to 4 years	272	8	157	3
Luo [301]	Zambia	1996	Adults	401	6	101	0
Megraud [302]	Algeria	1990	Infants	411	18	247	15

[a]Presented at the IV International Conference on *Campylobacter* infections (1987).

than 5 years old may be more than 100 times higher than the rate in developed nations.[70] The case-to-infection ratio is probably quite high and varies inversely according to age, suggesting that immunity is an important factor.[71,72] Furthermore, adults naturally and experimentally infected with *C. jejuni* were less susceptible to illness if they had preexisting antibody titers to *Campylobacter.*[73,74] *Campylobacter* infections are an important cause of childhood morbidity from watery diarrhea in developing countries.

5.2. Epidemic Behavior

In addition to endemic infection, outbreaks of disease associated with *Campylobacter* regularly occur in developed countries. Outbreaks do not occur where *Campylobacter* infections are hyperendemic, such as in many areas of the developing world, probably because of a high prevalence of postexposure immunity.[28] Outbreak investigations have provided much of the information about specific vehicles for transmission of infection (Section 6).

Investigations of outbreaks have shown that asymptomatic infection may occur in those exposed to an implicated vehicle[68,75,76] and that the spectrum of illness produced may be broad. Secondary spread of infection does occur in close contacts such as family members. In follow-up studies in Israel, 20 (10%) of 200 family contacts were found to excrete *Campylobacter*.[77] However, in most sporadic cases the source is not known and it is difficult to distinguish co-primary from secondary infections.

5.3. Geographic Distribution

Campylobacter infections are present in all parts of the world, as documented by studies of diarrheal disease on all inhabited continents (Tables 2 and 3). Isolations have been made from patients in tropical, temperate, and Arctic climates. Travelers who have acquired *Campylobacter* infections in every region of the world represent a considerable proportion of the total cases in Scandinavian countries.[78,79] Similarly, persons from developed countries who are living in developing countries are also at risk for *Campylobacter* infection.[80] In northern climates such as Canada[81] and Iceland,[82] *Campylobacter* infections are reported more commonly among persons in rural areas. In Ontario, Canada, the annual case rate was 80 per 100,000 in urban or nonfarm areas and 350–400 per 100,000 in farm areas where cases were often associated with raw milk ingestion.[81] High isolation rates in rural areas of Africa have been attributed to close association with domestic animals.[83]

The prevalence of *Campylobacter* infection is higher in developing than in developed areas (Table 3). *Campylobcter* infections appear to be increasing in developing countries and in some areas are surpassing *Shigella* and *E. coli* as causes of diarrhea.[84] Studies of healthy children in rural South Africa,[85] rural Bangladesh,[86,72] Central African Republic,[71] and The Gambia[87] found that asymptomatic infection of young children was prevalent. In The Gambia, 7.6% of asymptomatic children 2–4 years old were infected, and in both South Africa and Bangladesh, 40% of healthy children 9–24 months old were culture positive on a single determination. In a longitudinal study, *Campylobacter* infections were found in 41% of 127 children who were cultured biweekly from birth until 6 months of age.[71] As a consequence of continued and widespread exposure to campylobacters, in developing countries there is no difference in the isolation rate of *Campylobacter* from children with and without diarrhea. Furthermore, because infection and immunity are acquired early in life, *Campylobacter* infections are uncommon in adults in developing countries; no difference in isolation rates was observed among adults with and without diarrhea in Bangladesh.[88] After infection, the duration of excretion of *Campylobacter* is shorter (about 7 days) than it is among persons in developed countries. The high *Campylobacter* isolation rate among persons with diarrhea is caused by frequent transmission of organisms among the population rather than prolonged convalescent excretion.[89]

5.4. Temporal Distribution

In industrialized countries in temperate climates there is a definite increase in isolation rate during the hot summer months and a definite decrease during the cold winter months.[90,91] In Scandinavian countries there is another peak after winter holiday seasons resulting from infections in travelers.[78] In tropical, developing countries there is usually no seasonal variation.[72]

5.5. Age and Sex

In industrialized countries there is a bimodal age distribution. The largest peak occurs in children less than 5 years old and a second peak occurs in young adults 20–29 years old (Figure 1A). Among hospitalized patients, a third peak is observed among persons 60–80 years old.[29,79] In *Campylobacter* infections among children and young adults, males usually predominate[26] (Figure 1A), and among older persons the ratio is equal or in favor of females. In Colorado, for example, males predominate in all age groups under 40 and the greatest difference between sexes was in infants, where the male-to-female ratio was over 2:1.[90] The reason for the higher rate in males remains unexplained. In keeping with the second peak of isolation in young adults, *Campylobacter* is the predominant cause of diarrheal disease at American universities.[91] Eating under-cooked chicken and close association with cats are recognized risk factors among university students.[91,92]

In developing countries, isolation rates are highest among children less than 1 year old and do not increase again in young adults (Figure 1B). The highest isolation rate occurs in children 6–12 months old (Figure 1B inset). In developing countries, one to two *Campylobacter* infections per year for children under 5 years of age would be a conservative estimate. Serological surveys among healthy persons have shown a speak in *Campylobacter* IgG antibodies in children aged 1 year in Thailand and ages 2–4 years in Bangladesh.[28] Infection, even in infants, is frequently not associated with disease in areas of hyperendemicity.[71]

5.6. Occupation

There have been reports of infection immediately after occupational contact with cattle and sheep or with

beef, swine, or chicken carcasses.[93,94] At present, there is no information to suggest that hospital personnel are at increased risk of infection.

5.7. Other Factors

Campylobacter infections may also be sexually transmitted in homosexual men.[95] Two species, *Helicobacter cinaedi* and *H. fennelliae* have been isolated from homosexual men,[40,96] as have *C. fetus* subsp. *fetus*[97,98] and *C. hyointestinalis*. Homosexual men who have been infected with multiple etiologic agents appear to be at an increased risk of these *Campylobacter* species of low virulence. Prolonged or unusually severe *C. jejuni* infections have been recognized in patients with hypogammaglobulinemia or acquired immunodeficiency syndrome (AIDS) (Section 8.1). Most patients infected with *C. fetus* subsp. *fetus* have been debilitated or compromised by chronic disease or were at the extremes of age.[41,97,99] Since most *C. fetus* subsp. *fetus* infections are detected by blood culture, it is possible that only the severe end of the spectrum of disease is recognized. When *C. fetus* subsp. *fetus* was isolated from stool in a milk-borne outbreak, it caused a diarrheal disease indistinguishable from *C. jejuni*.[42]

5.8. Atypical *Campylobacter* Species

In places where antibiotic-free media or filtration methods are used, the proportion of non-*jejuni* campylobacters is >20% and in some countries approaches 50%[100–102] especially in tropical developing countries.. These organisms are increasingly recognized as human pathogens, perhaps in all populations but especially in immunocompromised persons (Table 1).[103–105]

C. fetus typically causes bacteremia or other extraintestinal infections in immunocompromised hosts.[106–109] This species also may cause uncomplicated diarrhea in immunocompetent persons.[100] *C. fetus* infections may be transmitted to humans via raw milk or other raw or undercooked foods of bovine or ovine origin.[42] *C. upsaliensis* is a catalase-negative, thermotolerant *Campylobacter* that causes diarrhea in young children; the organism has also been isolated from blood.[42,111] *C. upsaliensis* was first isolated from dogs, which are the principal reservoirs for this organism. In some parts of the world, such as South Africa and the Netherlands, more than 10% of all *Campylobacter* isolated are *C. upsaliensis*.[105,112] *C. upsaliensis* strains from different continents exhibit substantial differences in their genomic characteristics.[113] *C. hyointestinalis*, a causal agent of proliferative enteritis in swine, also has been associated with watery, nonbloody diarrhea in young children and in adults.[114,115] *C. lari* (formerly *C. laridis*) is found in 25% of healthy seagulls; this *Campylobacter* can produce acute diarrheal illness in young children and has been isolated from the blood of immunocompromised persons.[116,117]

Helicobacter cinaedi and *H. fennelliae* are a cause of enteritis and proctocolitis in homosexual men and sometimes also may cause bacteremia.[117–125] Malnourished children in developing countries also are susceptible to infection with these organisms.[120] Recent studies suggest these organisms may cause diarrheal disease in immunocompetent and heterosexual populations as well.[121] *Arcobacter cryaerophila* and *A. butzleri* were moved from the *Campylobacter* genus in 1991; both organisms can cause gastroenteritis.[122,123] *C. jejuni* subsp *doylei* has been isolated from the stools of children with diarrhea.[124] The organism also has been isolated from blood, usually in children with gastrointestinal symptoms, suggesting an intestinal source of infection. H2-requiring Campylobacters (*C. concisus, C. rectus, C. curvus, C. mucosalis*) have been associated with periodontal disease.[125] Their role as gastrointestinal pathogens has not been established.

6. Mechanisms and Routes of Transmission

Transmission of *Campylobacter* appears similar to that for other known enteric pathogens, i.e., transmission by way of contaminated food and water or by oral contact with fecal material from infected animals or humans. This information comes from investigation of several large and small outbreaks and from case–control studies of sporadic infections.

In developed countries, most *C. jejuni* infections are acquired through consumption and preparation of chicken. Microbiologic surveys have shown that chicken carcasses are nearly always contaminated with *Campylobacter.*[126] It is, therefore, perhaps not too surprising that in case–control studies of sporadic *C. jejuni* infections, up to 70% of cases are associated with eating chicken.[127–130] The greatest risk of infection comes from eating chicken prepared outside the home. Serological surveys indicate increased exposure to *Campylobacter* among poultry workers.[94] Other outbreak reports have implicated cross-contamination of foods in kitchens as a source of human infection and illness.[131,132]

Other foods of animal origin (e.g., beef, pork) may occasionally be related to *C. jejuni* infection.[133]Eating sausages was shown to be an important risk factor for sporadic cases of *Campylobacter* infection in Norway.[134] Nonmeat products such as mushrooms may be contaminated in the soil.[135]

In contrast to sporadic cases, outbreaks of *C. jejuni* infection are associated most commonly not with eating

chicken but with drinking raw milk.[136] From 1972 to 1990, 20 raw milk-associated outbreaks involving 458 people occurred among children and teenagers.[137] Fecal excretion of *Campylobacter* in milk cows and cattle is common and probably the usual source of contamination. *Campylobacter* infection of the bovine udder (mastitis) also may be a source of milk contamination. *Campylobacter* remains viable in refrigerated milk for 3 weeks and when ingested in milk may be protected from the effect of gastric acid.[36] As few as 500 *Campylobacter* cells are infective when given with milk.[61] Persons who regularly drink raw milk have elevated levels of antibodies to *Campylobacter* compared to age-matched persons who do not drink raw milk.[73] *C. fetus* subsp. *fetus* also has been associated with diarrheal disease after the ingestion of raw milk.[42] In the United Kingdom, pasteurized, bottled milk may be a source of *Campylobacter* infections because of contamination of the milk by birds (magpies and jackdaws) pecking the milk bottle tops.[37]

Waterborne outbreaks of *Campylobacter* infections have been reported in the United States, Europe, and Israel.[67,75,139–142] In the two largest outbreaks affecting 3,000 persons in Vermont[141] and 2,000 persons in central Sweden,[139] untreated surface water most likely contaminated drinking water systems. In smaller outbreaks at an English boarding school[140] and in a Florida town,[67] water in open-topped storage tanks became contaminated perhaps by feces from birds or rodents. During heavy rains, an untreated water reservoir in a Norwegian community was contaminated with sheep feces; 680 (66%) of the 1,021 community residents who drank the water developed symptomatic *Campylobacter* infection.[75] In a national park in the Rocky Mountains of the western United States, ingestion of untreated surface water was the most important risk factor for *Campylobacter* infection, which was found to be a more common cause of diarrhea than giardiasis.[141] In Colorado, ingestion of raw water is an important risk factor for sporadic cases.[143] *C. lari* was the probable cause of a waterborne outbreak among 162 construction workers in Canada when lake water was cross-connected to the main drinking water supply.[28] This outbreak provided convincing evidence that *C. lari* was a human diarrheal disease pathogen.

C. jejuni infections have been associated with foreign travel[144,145] and with exposure to dogs and cats.[146,147] Since the animal species with which humans come into contact most frequently—including dogs, cats, cattle, sheep, goats, chickens, turkeys, swine, horses, and rodents[148]—have been shown to excrete *C. jejuni* in their feces, the potential reservoir for human infection is enormous. Serotypes of *Campylobacter* isolated from animals are essentially the same as those isolated from humans.

As with other enteric infections, infected humans may be a reservoir for further transmission of infection, but *Campylobacter* is microaerophilic and sensitive to desiccation. Person-to-person transmission has been reported;[149] however, the index case has usually been a young child not yet toilet trained. There have been several reports of vertical transmission of *Campylobacter* infection from symptomatic or asymptomatic mothers to their neonates.[150,151] A contaminated bath plug may have caused an outbreak of *C. jejuni* infection among 11 children in the neonatal and pediatric intensive care units of a Dutch hospital.[152] Among 19 reported cases of premature labor, abortion, and perinatal sepsis due to *Campylobacter* species, *C. fetus* subsp. *fetus* was isolated in 9 and *C. jejuni/coli* in 10.[153] Considering that *C. jejuni* infection is many more times (ca. 500) more common than *C. fetus* subsp *fetus* infection, *C. fetus* must be considered to have a propensity for these complications.[41] Neonatal infections may be severe. A nosocomial outbreak of meningitis occurred in a neonatal nursery in France.[154] Transmission via infected food handlers or health care workers has been described but is rare.[155] There has been a report of laboratory-acquired infection,[156] but transmission to hospital personnel from patients or specimens in the hospital appears to be uncommon. Unlike *Shigella*, *Giardia*, or hepatitis A infections, there have been no outbreaks of *C. jejuni* infection reported in daycare centers or institutions for the mentally retarded.

7. Pathogenesis and Immunity

The minimal dose of *Campylobacter* that produces human infections is not known. A volunteer who ingested 10^6 organisms in milk became ill within 3 days;[153] however, infection has been induced with as few as 500 organisms.[61] In general, attack rates have been dose dependent in volunteer studies.[62,157] In a large milk-borne outbreak, almost all patients experienced the onset of illness within 2–7 days of drinking the contaminated milk.[158] For most pathogens, the length of the incubation period is inversely related to the dose ingested. The marked sensitivity of *Campylobacter* to gastric pH[36] suggests that it would take a high dose to infect 50–100% of subjects. The ID_{50} when given with sodium bicarbonate was 10^8.[62]

All campylobacters have a flagellum at one or both ends; the organisms' motility contributes to their ability to colonize and infect the intestinal mucosa.[159–161] Infection leads to multiplication of organisms in the intestine; patients shed 10^6–10^9 *Campylobacter*/g stool,[36] concentrations similar to those in *Salmonella* and *Shigella* infections. The sites of tissue injury include the jejunum, ileum, and

colon,[36] although whether the small or large intestine is more commonly affected is not known. In both organs, acute exudative and hemorrhagic inflammation may occur, and the appendix, mesenteric lymph nodes, and gallbladder may also be affected by the inflammatory process.[14] Patients with severe clinical manifestations frequently have colonic involvement. The pathological lesion may include infiltration of the lamina propria with acute and chronic inflammatory cells and destruction of epithelial glands with crypt abscess formation. This nonspecific colitis may mimic that seen in other types of infectious colitis, acute ulcerative colitis, or Crohn's disease. A pseudomembranous colitis has also been described.

A choleralike enterotoxin has been described by some researchers,[99,151,162,163] but others have been unable to reproduce these results.[74] More than 20 years after this toxin was first described, considerable evidence suggests that it does not exist. Enterotoxic activity is not found in fecal specimens from *C. jejuni*-infected persons.[165] Furthermore, unlike persons with cholera or those infected with enterotoxigenic *E. coli* persons infected with *C. jejuni* do not possess antibodies to the putative toxin. Even if the presence of an enterotoxin were proven, its biological relevance would be questionable since the biological lesion of *C. jejuni* enteritis is inflammatory enteritis. A Shiga-like cytotoxin is produced in vitro by a small number of strains but only at very low titers.[166] The biological significance of this toxin is also uncertain. Increasing evidence suggests that *C. jejuni* is locally invasive,[167–169] although not in high numbers, and that infection with this organism possibly involves novel mechanisms. A protein that distends epithelial cella and is cytolethal is expressed by many *C. jejuni* strains but its role in pathogenesis has not been fully defined.[170,171]

Although strains have different pathogenic properties,[151] as yet there is no well-defined association between the clinical illness and any virulence property. The presence of bacteremia in some patients and the finding of cellular infiltration in biopsy specimens from patients with *Campylobacter* colitis suggest that tissue invasion may be one pathogenetic mechanism. Experimental challenges both in monkeys[172] and in vitro[173–176] confirm the invasiveness of *C. jejuni.* The process of *C. jejuni* invasion is multifactorial. After invasion, cellular architecture is altered. Microtubule-dependent mechanisms may be important in the organism's ability to invade the intestinal epithelium.[177] The bacteria's flagellae also are important virulence factors because they promote the motility and chemotaxis needed for *C. jejuni* to colonize the intestinal tract.[173,178] In vivo passage favors flagellated cells;[179] intact flagellar synthesis genes are required for maximal *C. jejuni* invasiveness.[180,181] A cytolethal distending toxin may affect cell cycle kinetics.[45,79] A high-molecular-weight plasmid also enhances the invasive capabilities of *C.jejuni* virulence.[183,184] *C. jejuni* may adhere to epithelial cells,[185] which favors gut colonization. A superficial antigen (PEB1) that appears to be the major adhesion[186] is conserved among *C. jejuni* strains, is a target of the immune response,[187] and may represent a vaccine candidate.[188,189] Chemotaxis[191] and production of fimbriae[191] also are important in virulence.

In developed countries over 50% of patients with enteritis caused by *C. jejuni* have bloody diarrhea,[19] while in developing countries the proportion varies from 10 to 30%.[72,89] *Campylobacter* serotypes show little geographic variation and there is no other strain difference that can account for the difference in the severity of the illness. Immunity to *Campylobacter* infection, which is higher among persons in developing countries, may prevent illness or modify the illness to a less severe form. Hippurate-negative *Campylobacter* strains may be less virulent than *C. jejuni*, which is hippurate positive. In most surveys from developed countries, hippurate-negative strains account for a small proportion of the total number of *Campylobacter* isolates; however, *C. coli* accounts for 30–40% of strains isolated in Hong Kong[192] and the Central African Republic,[193] a sufficient number to make an impact on the observed spectrum of illness.

Most intestinal isolates of *C. jejuni* are killed by normal human serum (serum-sensitive), while extraintestinal isolates may be either serum sensitive or serum resistant.[194] Extraintestinal isolates from normal hosts are usually serum resistant; those from impaired hosts are serum sensitive as are the intestinal isolates.[41]Serum resistance in *C. jejuni* strains has been associated with the total amount of lipopolysaccharide (LPS) produced.[41,195] The LPS may serve as a type of capsule that protects the organism from the killing activity of serum.

Studies in Canada,[196] the United States,[197] the United Kingdom,[158] and Sweden[21] have shown the median duration of excretion to be 2–3 weeks from onset of symptoms. Almost all patients were culture negative by 2 months. In developing countries the duration of excretion is 7–10 days.[72,89] A longer excretion period was observed in Thailand among children less than 1 year old (14 days) compared to older children (8 days), suggesting that previous immunity may shorten the convalescent excretion period.[89]

Patients infected with *Campylobacter* develop specific serum antibodies against a variety of *Campylobacter* proteins and its LPS.[27,198,199] The response to LPS is both species and type specific.[200] The IgM antibodies rise and fall earlier than the IgG and do not reach the height of the IgG titer rise. Titers of both classes of antibodies appear to decline within

several months of infection.[27] Serum antibodies developed in response to infection with one *Campylobacter* strain are cross-reactive with heterologous strains.[201] In developing countries, infection rates, case-to-infection ratios, as well as the duration and magnitude of convalescent carriage decline with age.[29,202] IgG antibody titers to *Campylobacter* antigens peak in the first few years of life and then decline, while IgA antibody titers continue to increase throughout life;[28] repeated infections may stimulate specific IgA production, but have little effect on systemic IgG production. After acute infection, IgA appears in the serum more rapidly than IgG and is predominantly in polymeric form.[203] Intestinal antibodies have also been detected.[204] Uninfected individuals have low-titer antibody to specific organisms. Volunteers with elevated serum and intestinal antibody levels to *Campylobacter* proteins become infected but are protected from illness. Similarly, habitual raw milk drinkers were protected from illness when exposed to contaminated raw milk that led to illness in other persons.[73] Little is known about the role of the cellular immune response in the control of *C. jejuni* infections. However, the increased severity and duration of these infections among HIV-infected persons suggest that cell-mediated immunity does play some role.

8. Patterns of Host Response

8.1. Clinical Features

The consequences of infection with *C. jejuni* vary from asymptomatic excretion to death. *Campylobacter* is rarely isolated in the stools of random selected, asymptomatic persons. However, in the setting of an outbreak, the infection-to-illness ratio may be high. In one large outbreak, 25% of those infected were found to be asymptomatic.[158] In another large outbreak >50% of infected persons were asymptomatic.[205]

The most common presentation of *C. jejuni* infection is an acute enteritis. In general, the symptoms and signs of *Campylobacter* infection are not so distinctive that the physician would be able to distinguish it from illness caused by other enteric pathogens. At the mild end of the spectrum, symptoms may last for 24 hr and be indistinguishable from those seen with a viral gastroenteritis. Such patients usually do not seek medical attention; the occurrence of those cases has been detected only in outbreaks.[68] In industrialized nations, bloody diarrhea or the presence of fecal leukocytes is often a helpful clinical indication of *Campylobacter* infection.

Most information on the clinical features of *Campylobacter* infection is based on hospital- or laboratory-based studies and is thus biased toward those who are the most severely ill and who have diarrheal illnesses. In a multicenter study[19] in the United States where diarrhea was the criterion for stool culture, *Campylobacter* infection was associated with abdominal pain (79%), fever $\geq$ 37.8°C (74%), and a history of bloody diarrhea (46%). Among those 10–29 years old, 31% of persons with a history of fever and ten or more fecal leukocytes had *Campylobacter* enteritis. The abdominal pain has been so severe on occasion as to mimic acute appendicitis.[14,206] Fever has been noted in up to 80% of these patients, although children have been febrile less frequently; convulsions have been reported. A prodrome of constitutional complaints, chiefly fever, may be more prominent than the enteric symptoms.

Illness usually lasts less than 10 days, but up to 20% of the patients studied in the hospital-based series had a more prolonged and severe illness, with persistently high fever and frequently grossly bloody stools.[207] With bloody diarrhea and abdominal pain being common manifestations, many were thought to have an acute bout of inflammatory bowel disease or a surgical abdomen before *Campylobacter* infection was diagnosed.[206] *Campylobacter* colitis may mimic the early presentation of ulcerative colitis. *Campylobacter* infection may be a cause of exacerbation of inflammatory colitis, but there is no evidence that it plays a role in the etiology of these diseases. Endoscopic examination is often helpful in differentiating infectious from idiopathic types of colitis.[208] Cultures obtained by endoscopy at the site of mucosal ulceration may be positive when stool cultures are negative. *Campylobacter* colitis also may present as a surgical abdomen because of severe abdominal pain or bleeding. In Sweden, 11% of persons with *Campylobacter* enteritis required hospitalization;[209] in Norway, 13.3% of patients were hospitalized.[206]

C. jejuni bacteremia occurs in approximately 1.5 per 1,000 intestinal infections;[210] the rate is highest in the elderly and in immunodeficient persons.[211] In most patients, the bacteremia is associated with diarrhea and other gastroenterological symptoms. In contrast, *C. fetus* bacteremias are infrequently associated with diarrhea. Of all *Campylobacter* bacteremias, 89% are due to *C. jejuni* or *C. coli*; only 8.6% are due to *C. fetus.*[210]

Rarely, *Campylobacter* may infect the biliary tract, giving rise to cholecystitis, pancreatitis, or obstructive hepatitis.[82,212,213] It has been reported to cause splenic rupture.[214] It may cause peritonitis in patients receiving peritoneal dialysis,[215] and occasionally has been isolated from the urinary tract in women. *C. jejuni* infections in pregnant women are likely to be mild and self-limited; however, neonatal sepsis and death can occur if the woman is infected during her third trimester.[216]

A reactive arthritis has been reported after both symptomatic and asymptomatic *Campylobacter* infections.

Similar to *Shigella, Salmonella*, and *Yersinia* infection, this complication is associated with HLA-B27 in about 60% of the cases.[217] In Scandinavia, 1–2% of patients with *Campylobacter* enteritis develop arthritis beginning 4 days to 4 weeks after the onset of diarrhea.[218] Interestingly, in another Scandinavian study, 32 patients in a hospital for rheumatic diseases developed culture-confirmed *C. jejuni* infection during a hospital outbreak; none of these patients developed exacerbations of their existing conditions.[219]

During the past decade, *C. jejuni* infections have been implicated as triggers of the Guillain–Barré syndrome (GBS), an acute demyelinating disease of peripheral nerves. Both serological and cultural studies indicate that 20–40% of GBS patients are infected with *C. jejuni* in the 1–3 weeks before the onset of neurological symptoms.[30,31,220] There is no relation between the severity of gastrointestinal symptoms and the likelihood of developing GBS following infection with *C. jejuni*; in fact, even asymptomatic infections can trigger GBS.

Between 30 and 80% of *C. jejuni* isolates from GBS patients in the United States and Japan belong to LPS (O) type19.[30,221,222,223] This serotype is present in less than 3% of randomly selected *C. jejuni* strains reported to the CDC, and thus is overrepresented in patients with GBS. Antibody cross-reactivity between structures on the cell surface (including *C. jejuni* O:19 LPS) and glycolipids or myelin proteins may explain this association.[224]In South Africa, O-type 41 overrepresented among GBS-associated *C. jejuni* infections.[225]

In addition to sporadic cases of GBS in the United States, Japan, and other industrialized countries, *C. jejuni* infection has been implicated as a potential cause of acute motor axonal neuropathy (AMAN) in China and other developing nations. AMAN is clinically indistinguishable from GBS but affects the peripheral nerve axon and spares myelin. AMAN occurs in epidemics during the summer and fall and principally affects children living in rural areas of northern China.[226] Case–control studies showed that children with AMAN were more likely than controls to have serological evidence of preceding *C. jejuni* infection.[225] Interestingly, patients in the United Kingdom who developed GBS following *C. jejuni* infection also had axonal degeneration.[220]

Other reported but rare postinfectious complications of *C. jejuni* infections include hemolytic anemia, hemolytic–uremic syndrome, carditis, and encephalopathy.[227–229] Erythema nodosum and migrating urticaria also have been associated with *Campylobacter* infections.[79]

Illness due to *Campylobacter* infection is frequently self-limited and usually ends within 10 days; however, one or more relapses can occur. Those with more severe symptoms may have persistence of illness for 1 month or longer. Rarely, *Campylobacter* infection may cause mild, chronic diarrhea.[31,230,231] Illnesses are more likely to be prolonged and more severe in patients with HIV infection.[22,232–234] Among persons with AIDS, *C. jejuni* infections are reported almost 40 times as frequently as in the general population.[23] This excess of *C. jejuni* infections affects only infections in the later stages of HIV disease; patients with early HIV infection and relatively high CD4 counts are not unusually prone to *C. jejuni* infection or relapses of such infections.[235] Hypogammaglobulinemia patients develop severe persistent and relapsing infections due to *C. jejuni*.[236,237] A lack of opsonizing activity also predisposes individuals to bacteremia and other extraintestinal manifestations, including osteomyelitis, cellulitis, and meningitis.[238,239]

The most important principle of treatment of acute gastroenteritis due to *C. jejuni* infection is maintenance of hydration and electrolyte balance. In most cases, this can be accomplished by encouraging oral fluids. Campylobacters are susceptible in vitro to a wide variety of antimicrobial agents.[240] Because erythromycin is safe, easy to administer, and has less of an inhibitory effect on fecal flora than other antibiotics, it is the recommended therapy. However, most clinical trials performed in adults or children have not found that erythromycin significantly alters the clinical course of *Campylobacter* infection.[241–244] Only one study in Peru demonstrated that children with bloody diarrhea benefitted from erythromycin therapy if treatment was begun early in the course of illness.[60] Treatment of susceptible organisms shortens the duration of convalescent excretion, which in areas of poor sanitation may be useful. Other patients who may benefit from antimicrobial treatment include those with prolonged (>1 week) or worsening symptoms, high fevers, or bloody stools. HIV-infected or other immunocompromised persons also should receive antibodies for *Campylobacter* enteritis. Because infection with *C. jejuni* in pregnant women may have deleterious effects on the fetus, pregnant women also should receive antimicrobial treatment for *C. jejuni* infections.

Years of testing for antimicrobial resistance in many parts of the world has demonstrated that the resistance rate to erythromycin among *C. jejuni* isolates has changed very little,[245–248], although there is an upward trend in erythromycin resistance among *C. jejuni* isolates in Thailand[249] and Sweden.[250] At one time it appeared that fluoroquinolones such as ciprofloxacin had emerged as the treatment of choice for *Campylobacter* enteritis in particular and for acute bacterial diarrhea in general.[251] Unfortunately, as the use of fluoroquinolones has expanded (especially in food animals), the rate of resistance of campylobacters to these agents has increased.[252–255] Resistance rates to tetracycline also are increasing, especially

among *C. coli* isolates.[256–258] The newer macrolides, azithromycin and clarithromycin, have excellent in vitro activity against campylobacters[259–261] but provide little clinical advantage over erythromycin and are considerably more expensive.[262] Nearly all strains of *Campylobacter* are resistant to cephalosporins.[263] Gentamicin and imipenem are active against campylobacters, with resistance in fewer than 1% of strains. Therapy with these agents is indicated in persons with bacteremia and other extraintestinal suppurative infections. Because gentamicin is ineffective against *Campylobacter* in the gut, oral therapy also must be given.

8.2. Diagnosis

The diagnosis of *Campylobacter* infection is based on a positive stool culture or a positive blood culture. The frequency of bacteremia was low (about 1%) in one series of patients hospitalized for acute *Campylobacter* enteritis.[264] Not all commercial blood culture methods support the growth of *Campylobacter.*[265]

Serological diagnosis may be useful in industrialized countries where background asymptomatic infection rates are low. Serodiagnostic techniques may also be useful in determining the role of *Campylobacter* in abortion, postinfectious syndromes, inflammatory bowel disease, and other late manifestations of *Campylobacter* infection.

Serotyping and biotyping of *C. jejuni* and *C. coli* have been useful in epidemiological investigations to determine associated cases and the source of infection. Typing as well as PCR techniques for direct detection of organisms has been successful in research studies but has not yet been applied to the clinical setting.

9. Control and Prevention

Because poultry, livestock, pets, and wild animals are the major reservoir for these organisms, control is based on interruption of transmission to humans from animals, animal products, or environmental sources contaminated by animals. Transmission of *Campylobacter* infection to poultry flocks most likely occurs via environmental sources;[217] therefore, careful hygienic practices on farms may reduce contamination of flocks. Awareness of the necessity for hand washing after contact with animals or animal products and the importance of proper cooking and storage of poutry products—indeed *all* foods of animal origin—are as important for preventing *Campylobacter* infections as they are for *Salmonella* infections. Similarly, consuming only pasteurized milk and treated water is an important sanitary measure for the prevention of illness by individuals. The majority of *Campylobacter* outbreaks would not have occurred if not for the consumption of raw milk. HIV-infected and other immunedeficient persons should be particularly careful to avoid high-risk foods (such as unpasteurized dairy products or untreated surface water), as the consequences of infection may be more severe. Because many outbreaks and sporadic cases of *Campylobacter* infection are caused by injection of contaminated water, special care should be taken to prevent contamination of community drinking water supplies with animal or bird excrement or with untreated water. Travelers to developing countries and campers should be cautioned against drinking untreated water. Routine use of antibiotic prophylaxis to prevent infection is not recommended.

Control of nosocomial transmission from patients hospitalized with *Campylobacter* infection is another important consideration. As such "excretion precautions" are recommended for infected infants, children, and adults with fecal incontinence. In general, the bases for control of enteric infection in the hospital are sanitary disposal of excretions and contaminated linens, and hand washing by the patient and by staff members after contact with the patient, especially following contact with excreta. For *Campylobacter* infection, precautions should be maintained for the duration of the illness.

To date, there have been no reports of transmission of *Campylobacter* infection by asymptomatic excretors. Asymptomatically infected food handlers or hospital employees need not be excluded from work, but the need for hand washing after defecation should be stressed to these individuals. Recommendations are different when persons are infected with *Campylobacter* and continue to have diarrhea. Because of the known risk of transmission of other enteric pathogens from individuals with diarrhea, food handlers and symptomatic hospital employees with patient-care responsibilities should not be permitted to work until the diarrheal episode has ended. Because of the diversity of serotypes of *C. jejuni*, the possibility of vaccination for prevention of infection is limited. However, identification of group antigens could make immunization feasible.

10. Unresolved Problems

Epidemiological and clinical research during the last 10 years has done much to define the disease and the sources of infection. The priorities should be directed at public health prevention. First, we must learn how to produce poultry and livestock that are not infected with *Campylobacter*. This strategy would not eliminate *Campylobacter* because of the vast reservoir in wild animals and birds, but

it should decrease considerably the number of human infections. Efforts also should be directed at determining how to process meat so that it is not contaminated with animal feces containing campylobacters.

Cheaper and simpler diagnostic methods should be available so that a greater number of patients can be cultured for *Campylobacter*, which in turn might lead to better surveillance and more opportunities to investigate outbreaks to find additional modes of transmission. Efforts should be made to introduce simple and inexpensive isolation methods into all diagnostic microbiology laboratories. This should be combined with training in isolation techniques for technicians. Research on new diagnostic methods such as simple antigen detection kits and non-radioactive DNA probes should be pursued and a simple, standardized serotyping scheme should be widely available and routinely used.

The role of *C. jejuni* infection in triggering GBS was not understood until recent years. The pathogenesis of this association as well as possible prevention and control strategies needs to be explored. The pathogenesis of *Campylobacter* infection, the host mechanisms that allow most infections to be self-limited, and the occurrence and mediators of immunity are still not well understood. Research in the area of pathogenesis and immunity should continue and may help provide the basis for effective prophylaxis or intervention.

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CHAPTER 10

Chancroid

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1. Introduction

Chancroid is caused by *Haemophilus ducreyi*, a gram-negative bacterium. Chancroid, a genital ulcerative sexually transmitted disease, is characterized by painful genital ulcers and inguinal lymphadenitis. Like other genital ulcerative diseases (GUD), chancroid facilitates the transmission and acquisition of human immune deficiency virus (HIV).[26] The concern over the synergy between HIV infection and chancroid has renewed the interest to understand the pathogenesis of the disease and to develop new diagnostic methods.

2. Historical Background

The first differentiation between chancroid (soft chancre) and syphilis (hard chancre) was made by Bassereau in Paris in 1852.[8] However, it was in 1889 when Augusto Ducrey identified the causative organism by serial inoculation of the purulent material from genital ulcers into the skin of the forearm of three patients.[23] He was able to maintain serial ulcers through as many as 15 generations. However, Ducrey was unable to grow the organism on artificial media. There is controversy regarding the first person who cultured *H. ducreyi* on artificial media; however, the credit has been generally given to Bezancon.[11] Benzacon and colleagues fulfilled the Koch's postulates by experimental inoculation of the organism, infection, and re-isolation of the organism from humans. Later, the name *H. ducreyi* was established in recognition of Ducrey's work.

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3. Methodology

3.1. Sources of Morbidity and Mortality Data

The epidemiology of chancroid is not well known because few clinical laboratories perform diagnostic tests, and most of the available data are based on prevalence studies in STD clinics using convenience samples. In addition, culture methods are insensitive and diagnosis of chancroid in developing countries depends on clinical features of the disease. In the USA, the epidemiology of chancroid is described from the data compiled by the Centers for Disease Control and Prevention (CDC).[18] There are no reported fatalities from chancroid since the organism does not cause systemic disease.

3.2. Surveys

There are no meaningful population-based surveys of the presence of previous or subclinical disease. The majority of surveys are done in medical or sexually transmitted disease (STD) clinics.

4. Etiologic Patterns

Haemophilus ducreyi is a fastidious gram-negative bacillus which is a strict human pathogen with no known environmental or animal reservoir.[41] Based on growth requirements, biochemical reactions, and antigenic relatedness, *H. ducreyi* was classified initially in the genus *Haemophilus*.[41] However, rRNA analysis showed that *H. ducreyi* is not found in the Pasteurellacea cluster that includes *Haemophilus* sensu stricto species such as *Haemophilus influenzae* and *Haemophilus aegyptius*.[22] Instead, *H. ducreyi* is found in the *Actinobacillus*

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cluster (4B) of the Pasteurellaceae.[21] Biochemically, *H. ducreyi* requires X factor but not V factor for growth, and catalase negative and oxidase positive by the tetramethyl-*p*-phenylenediamine method.[35] *Haemophilus ducreyi* requires an enriched medium containing hemin and serum for growth.[59] The doubling time of *H. ducreyi* ranges from 1.8 to 4 h.[59] The organism usually grows after 48–72 h of incubation at 33–35°C in a high-humid CO_2-enriched environment. The colonies are usually gray to tan, translucent and can be pushed intact across the agar plate. Gram stain of these colonies shows clumping of *H. ducreyi* in long parallel strands with an appearance called "school of fish". The protein profiles and lipooligosaccharide (LOS) structures from various strains of *H. ducreyi* are generally well conserved; however, at least one strain, 33921 has a variant protein profile and LOS structure. Both the whole-cell lysate and the membrane proteins from strain 33921 were found to have altered positions on the gels, both in their apparent molecular weight and pI values compared to the prototypical strain 35000HP. Thus, strain 33921 has been suggested to be a member of a second class of strains, termed class II strains.[48]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

An estimated 6 million cases of chancroid occur yearly worldwide.[54] The epidemiology of chancroid is poorly understood in resource-poor countries with high prevalence of the disease due to the lack of diagnostic tests. Moreover, the epidemiology of chancroid is different between developed and developing countries. In resource-poor countries, the prevalence of chancroid in genital ulcerative diseases ranges between 23 and 56% based on multiplex polymerase chain reaction (M-PCR).[19,58] The incidence of chancroid may exceed that of syphilis as a cause of genital ulcerative diseases in Africa, Asia, and Latin America.[45,50]

In developed countries, chancroid occurs in sporadic outbreaks. In the USA, a total of 54 cases were reported in 2003, reflecting an increase from 38 cases in 2001.[18] However, the overall incidence had decreased from 2.04/100,000 in 1988[16] to 0.02/100,000 in 2002.[18] Most cases of chancroid in the USA were reported among minorities living in urban areas and in the south.[59] *Haemophilus ducreyi* was detected in 20 and 12% of ulcers from patients in Memphis and Chicago, respectively.[40] Similarly, chancroid occurs in sporadic outbreaks in Europe. In a study from Amsterdam, The Netherlands, *H. ducreyi* was detected in 0.9% (3 of 368) of attendees with at a clinic for sexually transmitted diseases [15]

5.2. The Roles of Race and Gender in Chancroid

In outbreaks of chancroid in the USA, *H. ducreyi* infection is more commonly found in African American and Hispanic males than other ethnic groups.[24] In experimental infection with *H. ducreyi*, a multiple logistic regression analysis showed no effect of age or ethnicity on pustule formation rates.[14] However, there were only a small number of observations on African American subjects, which precludes definite comment on the effect of ethnicity on the development of disease.[14]

In natural infections, the male-to-female ratio ranges from 3:1 to 25:1.[41] However, in the human model of *H. ducreyi* infection, males and females develop papules (become infected) at similar rates.[14] However, males develop pustules at a rate nearly threefold that of females, consistent with the epidemiology of natural infection.[14]

6. Mechanisms and Routes of Transmission

Chancroid is a sexually transmitted disease and *H. ducreyi* gains access to the skin through a breakdown in the epithelium. Extra-genital lesions occur secondary to autoinoculation of the organism.[59] Recently, there is a report of three cases of lower-limb ulceration in children caused by *H. ducreyi.* It was postulated that *H. ducreyi* may be a previously unrecognized cause of chronic skin ulceration. Close contact between those children and family members with lower-limb ulceration may have resulted in contamination of skin with wound exudate, allowing *H. ducreyi* to enter via breaks in the epithelium. However, additional epidemiological studies are needed to determine the likely mode of transmission and reservoir for *H. ducreyi* among this population.[61]

7. Pathogenesis and Immunity

7.1. Pathogenesis

Haemophilus ducreyi is highly infectious for humans; however, the infectious dose in natural disease is not known. The estimated rate of chancroid transmission in natural infection is 70%.[47] In the experimental human challenge model, an estimated delivered dose of 1–100 colony-forming units is sufficient to cause disease.[5,14] During the course of chancroid, *H. ducreyi* is found in pustules and in the dermis of experimental lesions.[9] In addition, the organism associates with collagen and fiber and has little association with keratinocytes, fibroblasts, T cells, Langerhans' cells, laminin, or fibronectin.[9] Thus, *H. ducreyi* remains extracellular and

resists phagocytosis. Moreover, *H. ducreyi* is chiefly found within the granulocytic infiltrate and colocalizes with neutrophils and fibrin at the ulcerative stage of disease.[10] In the human model of *H. ducreyi* infection, skin biopsies had an enrichment of T cells and macrophages after 48 h of infection and a neutrophil predominance by 7–9 days.[31] Moreover, macrophages in lesions had high levels of CCR5 and CXCR4 compared with peripheral blood cells.[31] Thus, the presence of up-regulated HIV-1 coreceptors in *H. ducreyi* -infected lesions may explain the increased acquisition of HIV-1 in patients with chancroid.

Many putative virulence determinants of *H. ducreyi* have been tested in the experimental model of human infection by evaluating isogenic mutants. Few mutants of *H. ducreyi* have been found to be attenuated in the human model and include those that did not express the ducreyi serum resistance A (DsrA) protein,[13] HgbA,[4] or peptidoglycan-associated lipoprotein (PAL).[27] DsrA mutant lacks an outer membrane protein, DsrA, and may have been killed by serum transudate in the wound. HgbA mutant may be unable to utilize hemoglobin from extravasated red blood cells and PAL mutant may be structurally unstable.[6] In addition, expression of the LspA1 and LspA2 proteins[32] and an intact *flp* gene cluster[53] are required for the virulence of *H. ducreyi.* The *lspA1 lspA2* double mutant does not inhibit phagocytosis and is attenuated in the human model of infection.[32] *Haemophilus ducreyi* associates with dermal collagen fibers within infected skin lesions and is facilitated by the presence of NcaA, an outer membrane protein.[28] Expression of *H. ducreyi* collagen-binding outer membrane protein NcaA is required for virulence in swine and human challenge models of chancroid.[28] An isogenic mutant of *H. ducreyi* lacking the *ncaA* gene is attenuated in the swine and human models of *H. ducreyi* infection.[28]

On the other hand, other isogenic mutants of *H. ducreyi* were virulent in the model. These mutants include glucosyltransferase mutant, the D-glycero-D-manno-heptosyltransferase mutant and a sialyltransferase mutant.[62,63] A fine tangled pili (FtpA), a hemolysin-deficient, and the major outer membrane protein (MOMP) mutants were also virulent in the model.[3,46,57] The reasons why these mutants were virulent in the human model of infection had been discussed by Spinola et al.[51]

7.2. Immunity

Natural disease does not reliably confer protection against subsequent infections with *H. ducreyi.* Patients had repeated *H. ducreyi* infections during two large outbreaks in the USA.[12,29] Similarly, in the human model of *H. ducreyi* infection, the rates of papule and pustule formation were similar in the reinfection and the naïve groups.[2]

On the other hand, spontaneous healing of an ulcer variant "chancre mou volant" was described in the pre-antibiotic era.[55] Additionally, spontaneous healing of chancroid ulcers was reported in later outbreaks in North America.[12,29] However, the exact mechanism for such healing is not known. In the human infection model, men are more likely to develop pustules, with an odds ratio of 2.8 (95% CI, 1.6–5.0)-fold, than women.[14] In reinfection experiments, some individuals repeatedly resolve all inoculated sites while others repeatedly form pustules.[2,52] These data suggest that some hosts are able to contain the infection with *H. ducreyi*.

8. Patterns of Host Response

8.1. Clinical Features

It is thought that *H. ducreyi* gain access to the skin through a break in the integrity of the epithelium. After an incubation period of 4–7 days, a papule forms and is followed by a pustule within 2–3 days.[49] However, during the course of natural infection, only a minority of patients recall the presence of a papule or pustule at the site of inoculation.[41] Pustules usually rupture 2–3 days later, forming ulcers. These ulcers are usually painful and have irregular edges.[41] The presence of multiple ulcers may occur in one half of patients.[47] If left untreated, chancroid ulcers heal slowly over the following weeks to months.[38] The presence of painful inguinal lymphadenopathy occurs in half of patients with chancroid.[49] These lymph nodes are usually unilateral and become fluctuant and form buboes. Chancroid usually affects the genital skin and mucosa, but may rarely present on extra-genital locations following auto-inoculation and may occur on the inner thighs, breast, or fingers. However, chancroid has not been reported to cause systemic disease. If left untreated, *H. ducreyi* may cause significant tissue destruction and heals slowly over several months with scar formation. Chancroid may lead to phimosis or penile autoamputation.[41] However, *H. ducreyi* has not been reported to cause bacteremia.

HIV-seropositive male patients with chancroid tend to have a greater number of genital ulcers than HIV-seronegative chancroid patients.[60] However, the histopathology of *H. ducreyi* ulcers in both HIV-seropositive and HIV-seronegative patients is identical.[36] More recently, in experimental infection with *H. ducreyi* in 11 HIV-infected persons, the papule and pustule formation rates were similar to those observed in HIV-seronegative historical control subjects. The cellular infiltrate in biopsy samples obtained

from the HIV-seropositive and HIV-seronegative subjects did not differ with respect to the percentage of leukocytes, neutrophils, macrophages, or T cells. Thus, experimental infections in HIV-seropositive and HIV-seronegative persons are clinically similar and do not cause local or augment systemic viral replication.[33]

8.2. Diagnosis

The diagnosis of chancroid is usually based on the clinical features of the disease and exclusion of syphilis and herpes simplex virus. However, in studies that diagnosed chancroid by polymerase chain reaction (PCR) based tests, the clinical diagnosis is neither sensitive nor specific. Gram stain of purulent discharge may show the characteristic trail-road gram-negative coccobacilli. However, Gram stain has a low sensitivity and specificity.[38] Isolation of *H. ducreyi* requires special media and should be incubated in a humid environment with 5% CO_2 at 33–35°C. Generally two media are required for the isolation of *H. ducreyi*.[59] The first medium contains gonococcal agar base, 2% bovine hemoglobin, and 5% fetal calf serum (GC-HgS), and the other contains Mueller–Hinton agar base with 5% chocolated horse blood (MH-HB). These media are supplemented with 1% CVA (ie, cephalothin, vancomycin, and amphotericin B) enrichment or 1% IsoVitaleX to provide L-glutamine and vancomycin.[38] The addition of vancomycin (3μg/mL) inhibits the growth of gram-positive commensal bacteria but may also inhibit the growth of *H. ducreyi*.[34] These culture media are not usually available in all microbiology laboratories and the sensitivity of cultures is about 75% compared to PCR-based tests.[43,44]

Serodiagnosis of chancroid relies on the detection of antibodies directed against whole-cell lysate, lipooligosaccharide (LOS), and a recombinant protein enzyme immunoassay using *H. ducreyi* proteins hemoglobin receptor A (HgbA) and D15. Compared to multiplex PCR (M-PCR), the adsorption enzyme immunoassay has a sensitivity of 53–71% and a specificity of 57–71%.[58] The enzyme immunoassay has a sensitivity of 48±59% and a specificity of 89–90%.[20] The recombinant protein enzyme immunoassay has a sensitivity of 100% and a specificity of 62% compared to M-PCR.[25] However, the main use of serodiagnosis is in seroprevalence studies since antibodies against *H. ducreyi* may persist for months after infection.[20]

Multiplex PCR (M-PCR) is considered the gold standard for the diagnosis of chancroid. M-PCR detects simultaneously DNA targets from *H. ducreyi*, *Treponema pallidum*, and herpes simplex viruses 1 and 2. Routine cultures for *H. ducreyi* have a sensitivity of approximately 75% compared to M-PCR.[42] Unfortunately, M-PCR is not readily available commercially.

9. Prevention, Control, and Treatment

9.1. Prevention and Control

Chancroid is usually very painful and lacks a non-human reservoir, thus *H. ducreyi* infections may be contained in a given population. For chancroid to be maintained in a given population, a high sex partner change rate is required.[54] Thus, chancroid will disappear unless it is present in a highly sexually active population. Multiple strategies have been used to control chancroid outbreaks. In Thailand, a multi-intervention plan that included promotion of condom use and presumptive treatment of commercial sex workers with antibiotics resulted in a 95% reduction (from 30,000 cases/year to less than 2,000 cases/year) of chancroid.[30] In Kenya, syndromic treatment of GUD, condom use, and education resulted in a 78% reduction in GUD in an African mining community.[54] In the human model of *H. ducreyi* infection, a single dose of azithromycin (1 g) prevents the development of infection for up to 2 months after weekly inoculations with *H. ducreyi*.[56] Currently, there is no available vaccine for chancroid. Expression of HgbA is necessary for *H. ducreyi* to survive and initiate disease in a human model of chancroid.[4] Recently, in a swine model of *H. ducreyi* infection, an experimental HgbA vaccine efficiently prevented chancroid. In that study, histological sections of immunized animals lacked typical microscopic features of chancroid, and no viable bacteria were recovered from inoculated sites of HgbA-immunized pigs.[1] Formaldehyde-treated *H. ducreyi* cytolethal distending toxin (HdCDT) elicited toxin-neutralizing antibodies titers 200 times higher than those elicited by the native toxin.[37] However, many studies are needed before a vaccine could be even tested in the human model of *H. ducreyi* infection.

9.2. Treatment

The current CDC recommendations for the treatment of chancroid are as follows: a single dosage of oral azithromycin (1 g), ciprofloxacin 500 mg orally twice a day for 3 days, erythromycin base 500 mg orally three times a day for 7 days, or a single intramuscular dose of ceftriaxone (250 mg).[17] Generally, the cure rate of chancroid with most of these regimens is >90%. The use of a single dose of ciprofloxacin was shown to be as effective as a 7-day course of erythromycin.[39]

Antibiotic treatment failures in HIV-seropositive patients with chancroid have been described for suboptimal regimens even in HIV seronegatives.[60] However,

single-dose azithromycin and ciprofloxacin regimens were shown to have similar efficacy in chancroid regardless of the HIV status of the patients.[7,39] Treatment failures in HIV-infected patients frequently reflect co-infection with herpes simplex virus. Thus, HIV-positive chancroid patients should be followed closely and monitored for co-infection.[17] Sex partners of chancroid patients should be evaluated and treated regardless of the absence of symptoms if they had sexual contact in the preceding 10 days of the patient's symptoms.[17]

10. Unresolved Problems

Some aspects of the pathogenesis of chancroid, especially the early stages of the disease, have been studied in the human model of infection. A recent study showed the localization of *H. ducreyi* in naturally acquired chancroid. However, further studies are needed to determine the relationship between *H. ducreyi* and the host in the ulcerative stages of the disease. Since the organism is extracellular and serum resistance is important for virulence, a vaccine that produces bactericidal antibodies or blocks nutrient uptake could be effective in prevention. The development of vaccines for the prevention of chancroid would have a great impact on the reduction of the rates of chancroid and HIV in areas of high prevalence for both diseases.

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12. Suggested Readings

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CHAPTER 11

Chlamydial Infections

Julius Schachter and E. Russell Alexander

1. Introduction

There are several quite different disease patterns that result from human infection with chlamydial organisms. Infection with *Chlamydia trachomatis* may result in trachoma, a variety of other syndromes that accompany ocular or genital infection, or lymphogranuloma venereum (LGV). *Chlamydia psittaci* has one human disease manifestation—psittacosis. *Chlamydia pneumoniae* causes respiratory disease and infection has been associated with coronary artery disease.

Trachoma causes a cicatrizing keratoconjunctivitis, and its sequelae of keratitis, pannus, entropion, and trichiasis occur in severe form in certain endemic areas, primarily the hot, arid parts of the world. Trachoma is the leading infectious cause of blindness.

From a genital reservoir, *C. trachomatis* is transmitted sexually to cause a variety of diseases (Table 1). In the adult female, disease manifestations include cervicitis, salpingitis, urethral syndrome, Bartholinitis, and postpartum endometritis. In the male, they include urethritis and epididymitis. (Both sexes may contract inclusion conjunctivitis and proctitis.) Infant chlamydial diseases include inclusion conjunctivitis of the newborn, pneumonia, and possibly bronchiolitis and otitis media. In the United States, in other Western countries, and in some developing countries where it has been studied, *C. trachomatis* is the most prevalent sexually transmitted bacterium. In infancy, it is the leading single cause of pneumonia in the first 6 months of life.

LGV is a systemic sexually transmitted disease with worldwide distribution, particularly prevalent in warmer climates. The bubonic form is of declining importance in the United States, although proctitis due to the agent is more often recognized.

Chlamydia psittaci causes an interstitial pneumonia or generalized toxic disease of children and adults that is transmitted from infected avian species. It has become an infrequent disease, except from occupational exposure or exposure to infected psittacine house pets.

Chlamydia pneumoniae causes a range of respiratory diseases from pharyngitis and otitis media to bronchitis and interstitial pneumonia. Clinically and, to a great extent, epidemiologically, the manifestations are very similar to those caused by *Mycoplasma pneumoniae*. From present knowledge it is exclusively a human disease.

2. Historical Background

The description of trachoma is ancient, trachoma being one of the earliest of human diseases to be recognized as a distinct clinical entity.[1] It was described in the Ebers papyrus (1500 BC). The name *trachoma* was first used by Dioscorides in 60 AD, and the stages of the disease were described by Galen a century later. From the Middle Eastern reservoir, it spread throughout Europe in many waves from the time of the Crusades to Napoleon, being known as both "Egyptian and military ophthalmia." In the last century, the disease has decreased in incidence in many of the temperate climates of the world. It has disappeared from Europe, for example, and is essentially gone from foci that existed in the central United States. Although sulfonamide treatment was introduced in 1938, and tetracyclines and macrolides in following decades, they cannot be credited with this change. Rather, it has appeared to follow improvements in the standard of living and of hygiene practices. Trachoma has persisted in hot, dry climates and is still a major cause of blindness in many developing countries.

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Table 1. Disease Manifestations of *Chlamydia*

Chlamydia trachomatis			
Trachoma			
Oculogenital diseases			
Adult, male or female:	Adult female:	Adult male:	Infant:
Conjunctivitis	Cervicitis	Urethritis	Conjunctivitis
Proctitis	Salpingitis	Epididymitis	Pneumonia
	Urethral syndrome	Arthritis (Reiter's syndrome)	Bronchiolitis
	Postpartum endometritis		Otitis media
Lymphogranuloma venereum			
Chlamydia psittaci			
Psittacosis			
Chlamydia pneumoniae			
Pneumonia			

In 1907, Halberstaedter and von Prowazek[2] described cytoplasmic inclusion bodies in epithelial scrapings from trachoma cases. They named them "chlamydozoa" or "mantle bodies" because reddish elementary particles appeared to be embedded in a blue matrix or mantle. Two years later, the same investigators reported inclusions in infants with nongonococcal ophthalmia neonatorum. Lindner[3] named this disease "inclusion blenorrhea" and reproduced ocular disease in nonhuman primates from a mother's genital secretion. Inclusions were demonstrated in female genital epithelium and in nongonococcal urethritis (NGU) scrapings. Material from each of these sources and from infant conjunctivitis was used to produce follicular conjunctivitis in monkeys. Thus, it was believed as early as 1910 that trachoma and ocular genital diseases were caused by similar agents, and the vertical transmission of these agents was postulated.

In 1911, Nicolle, Cuenod, and Blaisot[4] in Tunis passed trachoma secretions through Berkefeld V filters and succeeded in producing a conjunctivitis in an ape and a chimpanzee, established to be trachoma by transmission to human conjunctiva. In the 1930s, Julianelle[5] for trachoma and Thygeson[1] for inclusion conjunctivitis expanded these studies with the use of smaller filters. After Bedson had described the unique developmental cycle of the psittacosis agent, Thygeson recognized that the same cycle held true for the agent of inclusion conjunctivitis. Thus, Bedson[6], noting the morphological similarity of the developmental cycles of the agents, termed them members of the psittacosis-LGV group of atypical viruses. This concept was strengthened by the finding by Rake, Shaffer, and Thygeson[7] that they all shared a common complement-fixing antigen.

In the period 1930–1950, the clinical and epidemiological features of trachoma and inclusion conjunctivitis were described[5–8]—all on the basis of cytological morphological identification. In 1938, the first effective therapy of trachoma occurred with the introduction of sulfanilamides, although it was recognized early that these drugs did not kill the agents. In the search for in vitro isolation techniques for Chlamydiae, the chorioallantois of the embryonated hen's egg and a wide variety of tissue-culture systems were explored. These attempts failed, and *Chlamydia* were believed to be a virus with propensity for epithelial growth. Macchiavello in 1944 and Stewart in 1950 claimed organism growth in the yolk sac of embryonated eggs, but their findings could not be confirmed. It remained for T'ang et al.[9] in China in 1957 to clearly isolate the agent of trachoma in yolk sac treated with streptomycin to retard bacterial contamination and to demonstrate that this material caused inclusion-positive conjunctivitis in the monkey model. Collier and Sowa[10] confirmed this finding in African studies in The Gambia and, in addition, showed serological response to the agent in human sera. They also showed that the antigen responsible for seroconversion was common to the psittacosis and LGV groups of agents. An explosion of trachoma research followed, including studies in Saudi Arabia, Egypt, Taiwan, and San Francisco. As the trachoma agent was studied, it became clear that it was not a virus but a small bacterium that shared with viruses the property of obligate intracellular parasitism. The agent of inclusion conjunctivitis was isolated by Jones, Collier, and Smith[11], using yolk-sac techniques. In the period 1960–1980, the development of cell cultures for isolation[12] and microimmunofluorescence methods for antigen identification, immunotyping, and measurement of host response[13–14] has permitted clinical and epidemiological characteristics of *C. trachomatis* diseases to be expanded.

Psittacosis and LGV were first described clinically much later than trachoma [Jüergensen[15] and Hunter[16], respectively], but in both cases their real definition took place in the first half of the twentieth century. The understanding of the biology of the organisms developed more

rapidly than with the trachoma and inclusion-conjunctivitis agents, primarily because of their greater virulence and host range. In particular, their pathogenicity for mice and for the embryonated yolk sac permitted the development of isolation methods and of serological methods (a complement-fixation test was developed as early as 1930).[17] With the advent of tissue-culture methods, the exploration of the molecular biology of Chlamydiae has expanded considerably.[18,19]

Chlamydia pneumoniae disease was first described by Grayston, Kuo, Wang, and Altman.[20] The agent had been recovered from the eye of a primary school child in 1965 in a prospective study of trachoma in Taiwan. Two decades later antibody to this isolate was found in the serum of children and adults with respiratory disease.[20] Saikku's observation that antibody to this organism was associated with coronary artery disease led to a dramatic increase in research on *C. pneumoniae*.[21]

3. Methodology

3.1. Sources of Mortality Data

For none of the diseases caused by *Chlamydia* is there significant mortality, and the rare deaths associated with infant chlamydial pneumonitis or psittacosis are usually in persons with serious accompanying underlying disease. Fatal outcome has no importance in the epidemiology of these conditions.

3.2. Sources of Morbidity Data

Trachoma is not a reportable disease in the United States. For the last 50 years, the World Health Organization (WHO) and many national health agencies have accumulated data on trachoma occurrence by clinical surveys, for planning control efforts, but no continuing statistics on incidence are available. In the United States the genital chlamydial infection is reportable in all 50 states but access to diagnostic tests is not universal and accordingly there is considerable underreporting. Even so, *C. trachomatis* infection is the most common infection reported to the CDC.[22] The WHO considers it the most common sexually transmitted bacterial pathogen, with an estimate of about 90 million new infections each year.[23] Other estimates have been made from sexually transmitted disease clinics where the ratio of nongonococcal to gonococcal disease has been used, along with other prevalence data, to estimate annual incidence.[24,25] LGV is a reportable disease, but because of its rarity, data are usually available only in annual summaries published by the U.S. Public Health Service. Recent awareness of apparent outbreaks of LGV proctitis may change that.[26] Psittacosis is reportable, and data on incidence are published weekly in the *Morbidity and Mortality Weekly Report* and are summarized in *Surveillance Reports* by the Centers for Disease Control and Prevention (CDC). In addition, CDC accumulates data on the occurrence of psittacosis in pet birds or in fowl flocks when these animals are submitted to state laboratories for inspection. There are no data on the incidence of *C. pneumoniae* infections except for small studies done in selected populations. Seroepidemiological studies show that the infection is very common in many countries.

3.3. Surveys

Trachoma-control programs, often supported by the WHO, have depended on clinical surveys of trachoma prevalence and of trachoma sequelae, including blindness. The WHO has helped to standardize survey methods.[27] Most recently, these have been combined with more general surveys of visual acuity and blindness. Traditionally, such surveys do not utilize laboratory diagnostic methods. This is not necessary for trachoma, since the disease is well-defined clinically.

Data on incidence and prevention of genital chlamydial infections are available from research studies. These have included studies in STD clinics, prospective studies in pregnant women, studies of chlamydial genital carriage in normal populations, and surveys in infants.

There are no significant survey data for LGV, psittacosis, or *C. pneumoniae* infections in the United States.

3.4. Laboratory Diagnosis

3.4.1. Cytological Identification. For *C. trachomatis*, the organism can be identified in cytological scrapings or in tissue specimens by recognition of characteristic intracytoplasmic inclusions.[28] Such identification in conjunctival epithelial cells, and less frequently in cervical epithelial cells, constituted the only identification possible from the time when trachoma and oculogenital diseases were first recognized, at the turn of the century, until the organism was isolated in embryonated eggs in 1957.

This diagnostic approach has been supplanted by the use of tissue-culture (TC) isolation methods and by the use of modern nonculture diagnostic tests that detect chlamydial antigens or genes. Study of epithelial cell scrapings stained with Giemsa stain (the time-honored method) may still be

useful for some studies, or when other diagnostic tests are not available.

Cytology is a relatively insensitive way to diagnose chlamydial infection. For many years the most sensitive method for identification of *C. trachomatis* infection of the genital tract was isolation in TC. Antigen detection by direct fluorescent antibody (DFA) staining or by enzyme immunoassay (EIA) are somewhat less sensitive and specific, but are widely used.[29]

Direct methods of detection of chlamydial DNA have not been more sensitive than EIA procedures. However, the introduction of amplified DNA probe technology, such as the polymerase chain reaction (PCR) or ligase chain reaction (LCR), in the mid-1990s, revolutionized chlamydial diagnostics. These tests are highly specific and are more sensitive than any other diagnostic tests, including culture.[30] The broad-based use of nucleic acid amplification tests (NAATs) should provide wide access to accurate *C. trachomatis* diagnosis. The recognition that non-invasively collected specimens (first catch urines and vaginal swabs) can be used in NAATs has opened the door for population-based screening.

Chlamydia trachomatis can be recognized in tissue sections by Giemsa stain, IF methods, and electron microscopy. All these methods have been used in human disease and in experimental models. But here too, the NAATs have replaced these older technology.

Chlamydia trachomatis cannot be recognized cytologically with any consistency in bubo aspirates in LGV using any of the staining methods. Inclusions have been seen in biopsy material, but this is a poor method of identification. Similarly, *C. psittaci* is rarely recognized in cytological preparations and rarely in tissue secretions. FA methods and Giemsa stains have been used effectively in tissue secretions of experimental animal models of *C. psittaci* infection, but are not useful in routine human diagnosis. *Chlamydia pneumoniae* strains are relatively difficult to isolate and have been recovered both from the embryonated egg and from tissue culture. Home-brew NAATs have been widely used in research on *C. pneumoniae* and *C. psittaci* infections, but there are no commercially available tests.

3.4.2. Isolation

3.4.2.1. Chlamydia trachomatis. The method of choice for isolation of *C. trachomatis* is by cell culture. Cells used are McCoy cells, the HeLa-229 cell line, BHK-21 cells, or L-929 cells. A necessary step in cell cultivation of *C. trachomatis* strains other than LGV is centrifugation of the inoculum into the cells. In addition, the cells are treated in some manner to enhance infection; Gordon et al.,[12] who developed the modern cell culture technique, used irradiation of McCoy cells. Cycloheximide has been substituted for irradiation and cycloheximide-treated McCoy cells are the most widely used method at present.[31] Specimen material is inoculated into individual vials with cell sheets on coverslips within them or into wells of microtiter plates that have been implanted with cells. They can be examined at 48–96 h after inoculation. Intracytoplasmic inclusions are recognized by iodine stain, Giemsa stain, or FA techniques. The most commonly used stain is iodine, which permits rapid identification. Iodine stain will only identify *C. trachomatis*. Giemsa staining will permit identification of *C. psittaci* strains in cell cultures, but since this is usually not necessary, iodine stains are most widely used. FA stains will be more sensitive, and can be species or genus specific. The sensitivity of the culture methods is enhanced by one additional passage through cells. LGV strains may be readily isolated in the same system as for the trachoma and oculogenital strains. They grow more readily in cell culture and do not require cell pretreatment or centrifugation.

Isolation of *C. trachomatis* from yolk sac was historically the first method of agent recovery. However, it is far less sensitive than TC, and thus is no longer used. Mouse inoculation is another possible method for LGV isolation. Intracranial inoculation is more effective than intranasal instillation, but both are inferior to yolk-sac inoculation. Trachoma and inclusion-conjunctivitis strains will not grow efficiently in mice.

3.4.2.2. Chlamydia psittaci. These agents will grow in cell culture, in yolk sac, and in mice, by a variety of routes of inoculation. Cultivation in yolk sac or embryonated chick egg is most commonly used in laboratories that are accustomed to this method, as is true of mouse inoculation. This may be accomplished by intraperitoneal, intracerebral, or intranasal inoculation. Since tissue-culture methods are now more widely available because of greater interest in *C. trachomatis* infection, they will be more widely used. In experienced laboratories, the methods have equal sensitivities. Care must be taken with any of these methods when attempting to recover *C. psittaci* strains, since they are more likely to result in accidental laboratory infections and in cross-contamination.

3.4.2.3. Chlamydia pneumoniae. These strains have been isolated from the yolk sac of embryonated eggs, and initially from HeLa cell TC. HL and Hep2 cells have been found to be preferable.[32,33] *Chlamydia pneumoniae* antigen in respiratory tract specimens or isolation systems can be demonstrated with species-specific antibody which is commercially available.

3.4.3. Antigen and Nucleic Acid Probes. In recent years, nonculture diagnostic methods have been evaluated and widely used. The first were direct fluorescent antibody smear (DFA), enzyme immunoassay (EIA), followed by

direct nucleic acid hybridization methods. None of these tests performs nearly as well as the NAATs.

Direct nucleic acid probes are still commercially available, and retain their popularity because they are less expensive than NAATs. They are less sensitive than culture, although they are highly specific.[34] In contrast, the NAAT technology, exemplified by PCR, TMA, and SDA (polymerase chain reaction, transcription-mediated amplification, and strand displacement assay), is far more sensitive than TC and it is extremely specific.[30,35,36] The chlamydial genes can be detected in epithelial cell specimens and in male and female urines.[37–39] The potential for widespread use of noninvasive screening tests is an important option offered by this technology.

All these tests provide ease of transport, automated batch processing, and the ability to diagnose both chlamydial and gonococcal diseases on the same specimen. As with antigen identification methods, there is the drawback that no organism is available for further identification (e.g., serological markers or testing for antibiotic sensitivity). Also, NAATs are more expensive than culture.

3.4.4. Serological and Immunological Diagnostic Methods. *Chlamydia trachomatis*. Although a variety of serological methods have been used in the past, the FA method, first developed by Bernkopf and then by McComb and Nichols, has been simplified and standardized in an indirect microimmunofluorescence (MIF) test by Wang and Grayston. At present, this is the most commonly used test for evaluation of host response to trachoma or to oculogenital *C. trachomatis* agents.[13] For LGV, the complement-fixation (CF) test can be used, although the MIF test remains more sensitive and specific.

The MIF test employs yolk-sac- or cell-culture-grown *C. trachomatis* of all known immunotypes as antigen. Antigen dots are placed on a slide in a specific pattern. They are attached to the slide in a diluted yolk-sac suspension. Serial dilutions of serum, tears, or local secretions are applied followed by fluorescein-conjugated anti-human globulin. An obvious advantage of the test is that the conjugate may be prepared against any immunoglobulin class, thus permitting titration of IgG-, IgM-, or IgA-specific antibody. A counterstain is added to the conjugate to permit clearer identification of specific fluorescence in the dots to which antibody-containing serum has been applied. If differentiation among antibodies to specific immunotypes is sought, the antigens can be evaluated separately. For many purposes, antigen pools can be used.

A CF test is available to measure host response to any chlamydial infection.[40] It is still the most commonly used test for psittacosis and LGV, but it is too insensitive for trachoma or oculogenital infections. Usually, the antigen for the CF is obtained from a yolk-sac-grown LGV strain and is prepared by boiling and treatment with phenol. For *C. pneumoniae* strains, the CF test is intermediate in sensitivity. A CF response can be recognized in approximately one-half of cases showing response by MIF.[20] Many other serological tests have been evaluated and rejected.

For many years, skin-test hypersensitivity to an LGV antigen (prepared from bubo pus) was used for diagnosis of LGV (Frei test).[41] Before more sensitive and specific antibody measurements were available, it was a useful diagnostic aid. However, because cases of LGV are usually not seen early, conversion from negative to positive is rarely observed. In addition, the possibility that positive skin tests merely reflect earlier infections due to other Chlamydiae, along with the relative insensitivity of the test and the difficulty in standardizing antigen, have rendered the test obsolete.

Another immunological method of importance to epidemiological studies is the immunotyping of *C. trachomatis* organisms. Earlier, using a method that employed the protection from type-specific toxic death resulting from massive intravenous inoculation in mice (a method adopted from analogous methods in rickettsial research), it was learned that there were specific immunotypes of *C. trachomatis*. Type-specific immunity could be demonstrated and it appeared that there was some relationship of immunotype to broad disease categories.[42] Wang et al.[13] subsequently showed that a far simpler method of immunotyping could be developed using an MIF method. By this method, 12 immunotypes were shown to be associated with trachoma and oculogenital disease, and another three with LGV. More subtypes have continued to be identified. No such immunotyping method has been rigorously applied to *C. psittaci* but specific immunotypes do exist. For *C. pneumoniae*, only one type has been shown. Immunotyping of *C. trachomatis* strains has no broad diagnostic significance other than the differentiation of LGV and oculogenital strains, but immunotyping can be used as a marker of strains in epidemiological investigations.

With the increased ability to sequence DNA, it is now more common to identify genotypes rather than serotypes.

4. Biological Characteristics of the Organism

Chlamydiae are obligate intracellular bacteria. They were thought to be viruses for many years and were termed *Bedsonia* or *Miyagawanella*. However, it became clear that they had a complex cell wall (similar to gram-negative bacteria in composition), both DNA and RNA, prokaryotic ribosomes, and metabolic enzymes that would permit independent existence, except that they lack energy-production

mechanisms. Moulder[43] has thus termed the Chlamydiae "energy parasites" and credited their obligate intracellular parasitism to this trait.

The other unusual characteristic of the Chlamydiae is their reproductive cycle (Figure 1). The extracellular form of the organism is the elementary body, and it alone is infectious. Following attachment, the elementary body enters the host cell, apparently by a receptor-mediated endocytosis process. If the particles are live, or do not have antibody attached, they prevent lysosomal fusion and replicate in the phagosome. Within 6–8 h of ingestion, the elementary body is reorganized into a reticulate body (sometimes called an initial body). This larger, thin-walled body diverts the host cell's synthetic functions for its own metabolic purpose and proceeds to divide by binary fission. In this stage, the reticulate bodies are not infectious. After 18–24 h, the reticulate bodies become reorganized again into elementary bodies (and thus become infectious again). Subsequently, elementary bodies may disrupt and exit the host cell to infect new cells. The full cycle takes about 40 h for LGV strains, and 72–96 h for the trachoma biovar. These intracytoplasmic collections of elementary bodies constitute the inclusions that may be seen in cytological smears stained by Giemsa or immunofluorescence methods.

Although all Chlamydiae share a genus-specific antigen, there is little DNA homology between *C. trachomatis*, *C. psittaci*, and *C. pneumoniae* strains.[45] The three species of Chlamydiae are distinguished by several features. The elementary body of *C. pneumoniae* is pear shaped with a large periplasmic space, whereas the elementary bodies of the other two species are round with little or no periplasmic space. The inclusions of *C. trachomatis* accumulate glycogen and thus stain with iodine, whereas the inclusions of *C. psittaci* and *C. pneumoniae* do not. The inclusions of *C. trachomatis* are more compact than those of *C. psittaci* and *C. pneumoniae*. A fourth difference is their response to sulfonamides: *C. trachomatis* is sensitive and *C. psittaci* and *C. pneumoniae* are not.

Chlamydia trachomatis can be further subdivided into strains that cause LGV and strains that cause oculogenital infections. They differ significantly in their biological activity. LGV strains (three serotypes) are more invasive and

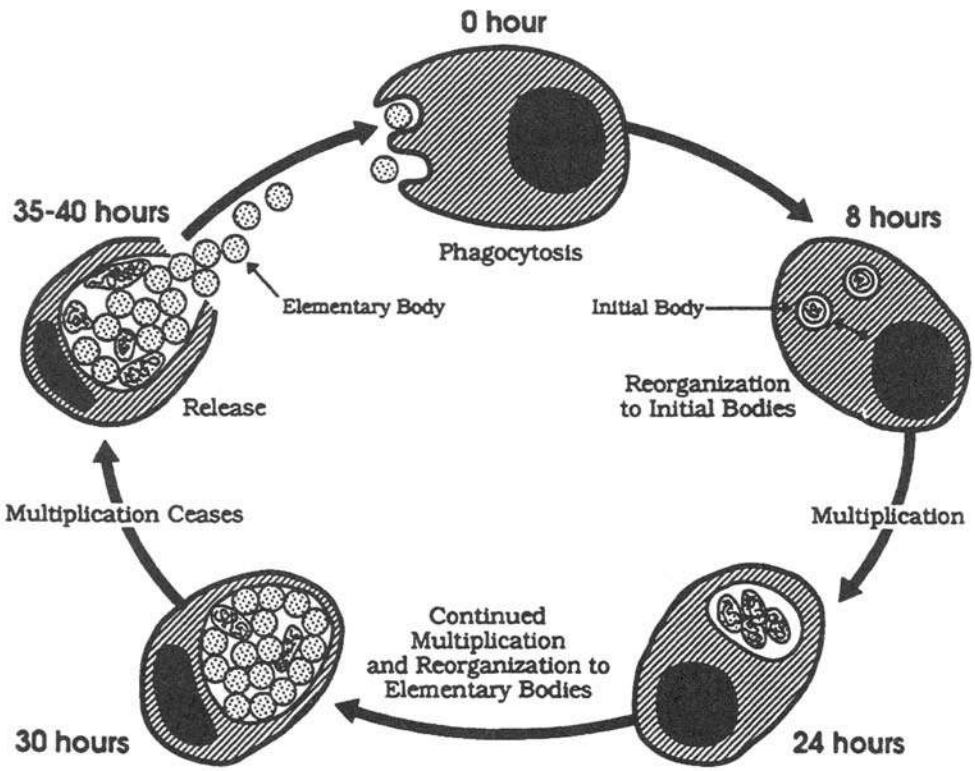


Figure 1. Life cycle of chlamydial organisms. The cycle begins when small elementary bodies infect the host cell by inducing active phagocytosis. During the next 8 h, they reorganize into the larger reticulated initial bodies, which then divert the cell's synthetic functions to their own metabolic needs, and begin to multiply by binary fission. About 24 h after infection, the daughter organisms begin reorganizing into infectious elementary bodies. At about 30 h, multiplication ceases, and by 35–40 h, the disrupted host cell dies, releasing new elementary bodies that can infect other host cells and thus continue the cycle.[44]

can invade many tissues in addition to epithelial cells (e.g., lymph-node invasion, forming the characteristic bubo). This property permits more active cell-to-cell transmission in cell culture and the use of animal inoculation (e.g., mouse) for diagnostic purposes. The oculogenital strains, comprising the trachoma biovar, consist of 12 serotypes. They are not readily invasive in tissue culture and in vivo grow only in columnar and squamocolumnar epithelial cells (conjunctivae, respiratory tract, urethra, cervix, and rectal mucosa).

Chlamydia psittaci will survive for long periods (years) in tissues frozen at –20°C and can persist in dry bird feces for months. Protein (e.g., serum albumin) will aid stability of *Chlamydia*. *Chlamydia trachomatis* strains will lose infectivity within 48 h at room temperature. They are heat-labile, being inactivated in minutes at 56°C. They are inactivated by common disinfectants (e.g., formalin, phenol).

5. Descriptive Epidemiology

5.1. Trachoma

5.1.1. Prevalence and Incidence. Prevalence surveys of trachoma in endemic countries are repeated frequently for control purposes. In hyperendemic areas, the prevalence of trachoma is essentially 100% by the second or third year of life.[46] Active disease, however, is most common in children, who constitute the reservoir of the disease. By adult life, active infection becomes infrequent, although the sequelae of the disease continue to produce the damaging effects that result in visual defect and blindness. In such areas, trachoma constitutes the major cause of preventable blindness.

In areas of moderate endemicity, infection and disease occur later, often beginning at school entry and going through young adult life.[47] The disease is milder in such settings and sequelae are mild, rarely leading to blindness. Often, within such areas, microfoci of severe disease will persist in certain family groups, where a full range of severity will be found.

The United States represents a nonendemic area where trachoma persists only in isolated population segments. As in the hyperendemic areas, this disease status is constantly changing as betterment of living standards results in decreasing prevalence. Thus, in southwestern American Indian populations 40 years ago, 10% of Indian schoolchildren were found to have active trachoma at school entrance.[48] Even as active disease is disappearing, sequelae of trachoma can still be seen in the majority of the older population segment.

5.1.2. Epidemic Behavior and Contagiousness. Trachoma is never seen in epidemic form, although perhaps it could return to susceptible populations under disaster conditions if disruption were to result in marked disturbance of hygienic practices. In hyperendemic areas, it persists and is transmitted by a variety of practices that permit eye-to-eye transmission. This may include the use of common washing utensils or the use of eye drops. The availability of water for washing will greatly affect its transmission. The transmission is potentiated by a variety of ecological factors. In particular, the presence of flies amplifies the transmission of disease. The occurrence of other bacterial eye infections (also transmitted by flies) and the effect of dust on eye irritation also facilitate active infection. When trachoma is reintroduced in nonendemic areas, it is not contagious as long as the hygienic and socioeconomic conditions do not facilitate the transmission.

5.1.3. Geographic Distribution. The trachoma-hyperendemic areas of the world are North Africa and sub-Saharan Africa, the Middle East, drier regions of the Indian subcontinent, and Southeast Asia. Foci of trachoma persist in Australia, the South Pacific, and Latin America.

Within the United States, there are no remaining areas of endemicity. At the turn of the century, there was an endemic focus in the south central United States (extending to Texas). A hospital in Missouri devoted to the treatment of trachoma was closed only in 1950. Similar disappearance of endemic trachoma occurred in Europe, Russia, and the Mediterranean in the same period.

5.1.4. Temporal Distribution. Throughout the world, trachoma has vanished as standards of living improve. Thus, the trends are usually ones of decrease. Within many endemic areas, there are seasonal changes that closely follow the seasonal increase in fly prevalence and the accompanying bacterial conjunctivitis. In some sites (e.g., northern India), two such peaks may occur each year. Such changes will not affect the prevalence of chronic trachoma or the blinding sequelae.

5.1.5. Age. The relationship to age is discussed in Section 5.1.1.

5.1.6. Sex. In hyperendemic areas, there is no sex differential in young children—the reservoir of active trachoma. In adults, reinfection disease occurs more frequently in women taking care of children, and they may also have a higher prevalence of blinding sequelae.

5.1.7. Race. There is no evidence for racial differences.

5.1.8. Occupation. There are no occupational factors that cannot be related to socioeconomic factors.

5.1.9. Socioeconomic Factors. These are predominant epidemiological factors. The important aspects appear to be water availability, fly occurrence, health and hygienic practices, and crowding. Face washing has been identified as

a protective behavior.[49] Studies in Saudi Arabia by Nichols et al.[50] depicted the decrease of trachoma occurring with the creation of small towns replacing rural foci where trachoma had been hyperendemic. Striking differences could be shown among cities, small towns, and oasis villages. In the United States, as in many other countries, trachoma has vanished with improvement in the standard of living.

5.1.10. Other Factors. Although nutritional factors have been questioned as a possible contributing factor, there is no clear evidence that such is the case, and the factors of water shortage, exposure to bacterial conjunctivitis, fly transmission, and the amplifying factor of dust irritation appear to be most important. Jones[46] summarizes these conditions as those that contribute to "ocular promiscuity" (in contrast to the amplifying factors of "sexual promiscuity" for genital *C. trachomatis* infections) (also see Section 6.1).

5.2. Oculogenital Diseases

5.2.1. Prevalence and Incidence. *Chlamydia trachomatis* infections are currently the most common infections reported to the Centers for Disease Control and Prevention (CDC). As of the year 2000, all 50 states and the District of Columbia required reporting of diagnosed chlamydia cases. In the year 2004, 929,462 chlamydial infections were reported to the CDC.[22] This compares to approximately 335,000 cases of gonorrhea being reported to the CDC in that same year. The rate of chlamydial infections in women in the United States increased from approximately 78 to more than 466 per 100,000 of the population in the period 1987–2003. This increase does not actually reflect real increases in infections, but the widening base for chlamydia screening and then more recently in the increased use of nucleic acid amplification tests (NAATs), which are sufficiently more sensitive than other types of diagnostic tests to be responsible for an increased detection rate. The general assumption is that the use of any NAAT will detect about 30% more infections when screening asymptomatic individuals. Forty-four states have chlamydial infection rates greater than 300 per 100,000 of the population. The prevalence in women 15–24 years of age and attending family planning clinics was approximately 6% (range approximately 3–19%). The range reflects differences across states. There have been programs involving the screening and treatment of those who have been found to be infected that have been in place for years, and these programs have had modest effects in decreasing the prevalence of infection in the clinic-attending populations. There is, however, still a relatively high prevalence in the population and in some areas minor increases have been seen. In prenatal clinics, infection rates in 15- to 24-year-old women had a median of 7.4% (range 2–20%).

The infection rate in men is approximately 1/6 the rate that is seen in women. This is largely a reflection of the lack of screening in male populations. Most male infections are diagnosed in symptomatic men, whereas most infections in women are in the asymptomatic.

In 34 states, chlamydia screening is being done in correction facilities. A number of different populations have screening for *C. trachomatis* on an ongoing basis. For example, approximately 20,000 females entering the national job training program have been screened for chlamydia each year since 1990. In 2003, the overall chlamydial prevalence in females entering the national job training program was 9.9%; for men the overall infection rate was 8%.

The introduction of nucleic acid amplification tests and the ability to test non-invasive specimen types have allowed epidemiologists to conduct population-based screening for chlamydial infections. While a fair amount of data has been generated by the CDC, the prevalence of *C. trachomatis* genital infection in the US population at large is unknown. Using urine-based screening, Mertz and colleagues[51] conducted a pilot study as part of the National Health and Nutrition Examination Survey III (NHANES III). The prevalence detected was relatively high in this stratified, multistate probability sample of the US population. Prevalence was 7% in non-Hispanic Blacks and 2% in non-Hispanic Whites. Prevalence was higher in females than in males in all subgroups. In the 15- to 19-year-old women (who represent the highest prevalence populations in essentially all studies), the prevalence was 13% in non-Hispanic Blacks, 11% in Mexican-Americans, and 5% in non-Hispanic Whites.

5.2.1.1. Adult Inclusion Conjunctivitis. No incidence or prevalence data in either males or females are available for this illness.

5.2.1.2. Cervicitis. Overall population estimates for chlamydial infection are difficult to obtain. However, figures have been obtained for different subgroups. Among pregnant women enrolling for prenatal care, 3–25% have been found to be infected, depending on the group examined. In venereal disease clinics,[52] up to 30% of symptomatic women have cervical chalmydiae. The prevalence in asymptomatic partners of males with urethritis may be as high as 50%. In asymptomatic nonpregnant college students, chalmydiae were found in the cervixes of 5%. In screening women in family planning clinics, infection rates have ranged from about 2–3% in higher socioeconomic settings to 30% in inner-city populations. Up to 70% of the chlamydial infections in these settings have been clinically inapparent.[53]

Etiology in cervicitis is more difficult to define, since multiple organisms may be involved. However, chalmydiae

have been isolated from 34 to 63% of women with clinical cervicitis.[52]

5.2.1.3. Salpingitis. In the United States, over 200,000 women per year are hospitalized for active pelvic inflammatory disease, and it is estimated that another million are treated as outpatients. If tubal cultures taken at laparoscopy are regarded as identifying the etiological organism, then *C. trachomatis* causes approximately 20–40% of salpingitis in this country and almost 60% in Sweden, where it has become an uncommon disease.[54] Serological data have also implicated *C. trachomatis* as a causal agent in peritonitis or perihepatitis or both (Fitz-Hugh–Curtis syndrome). Silent salpingitis is more common, and there is evidence that many women with asymptomatic cervical infection actually have upper genital tract disease.[55]

5.2.1.4. Female Urethral Syndrome. The prevalence of acute urethral syndrome is unknown. An estimated 5 million women per year in the United States are seen for symptoms suggestive of urinary tract infection. As many as 30% of these *may* have acute urethral syndrome (acute dysuria, frequent urination, "sterile" urine). Studies in select populations suggest that approximately 30% of the cases of acute urethral syndrome are due to *C. trachomatis*.[52] This has only been shown with young women.

5.2.1.5. Postpartum Endometritis. In one study, seven (22%) of 32 women with *C. trachomatis* cervical infection during pregnancy developed late postpartum endometritis (48 h, 6 weeks postdelivery).[56] In other studies the association has been less or not found.

5.2.1.6. Male Urethritis. In the United States, more than half the estimated 2 million cases of acute urethritis are of nongonococcal etiology. *Chlamydia trachomatis* causes 30–50% of these cases.[57] In some populations, e.g., student health clinics, *C. trachomatis* is far more common than gonococcus as a cause of urethritis.[58]

5.2.1.7. Epididymitis. This severe complication of acute urethritis occurs in approximately 500,000 men per year in the United States. Roughly half these cases are caused by *C. trachomatis*.[58]

5.2.1.8. Arthritis (Reiter's Syndrome). In studies in the United Kingdom, it has been estimated that Reiter's syndrome occurs in about 1% of NGU.[59] An estimate from Finland is 2%.[60] Sexually acquired reactive arthritis (see Section 8.2.1.h) as a complication of NGU without the classic triad of Reiter's syndrome occurs four times more frequently.[47]

5.2.1.9. Infant Inclusion Conjunctivitis and Pneumonia. Approximately 20–30% of infants exposed to *C. trachomatis* in the birth canal will develop conjunctivitis and 10–15%, pneumonia.[52,61] If a conservative figure of 5% maternal carriage rate is used, 3.0 million babies born each year in the United States would yield 45,000 cases of inclusion conjunctivitis and 20,000 cases of chlamydial pneumonia. *Chlamydia trachomatis* has been estimated to cause 20–30% of all pneumonia in infants less than 6 months of age ill enough to require hospitalization.

5.2.2. Epidemic Behavior and Contagiousness. The chlamydial oculogenital diseases behave like other STDs. The increasing incidence and prevalence of chlamydial infection probably paralleled that of gonorrhea, but as gonococcal infection rates have declined in recent years chlamydial infection rates have remained high and may reasonably be termed epidemic at this time.

In the United Kingdom and Sweden, where both gonococcal urethritis and NGU are reportable, the incidence of both diseases rose rapidly from the early 1960s. In fact, the start of the epidemic increase in Sweden preceded the start in the United States by about 4 years. The rest of the curve is very much the same as the United States for gonorrhea. In the mid-1970s, Sweden saw a plateau for the incidence of gonorrhea (the United States experienced the same phenomenon in 1978). In Sweden, the incidence of chlamydial infections has decreased markedly. Chlamydial infections are probably as contagious as gonorrheal infections, but studies to evaluate this have not been done. Reinfections are common, particularly among adolescents. After several years of reduction in prevalence it appears that many regions are experiencing a plateauing, or a slight increase in prevalence of cervical chlamydial infection.[22]

Chlamydia and gonorrhea occur together frequently. *Chlamydia trachomatis* may be recovered from 20 to 30% of men with gonorrhea. Postgonococcal urethritis is now recognized as a chlamydial infection persisting after successful treatment for gonorrhea. Chlamydial and gonococcal infections may occur together in the cervix (35–45% double infection is not uncommon) and inadequate treatment may result in salpingitis.

Chlamydial conjunctivitis may be transmitted from infected persons by contact. There is no evidence that chlamydial pneumonia of infants can be transmitted in any other way than at birth.

5.2.3. Geographic Distribution. The agent and its various clinical pictures have a worldwide prevalence; it has been identified in all populations in which STDs have been examined.

5.2.4. Temporal Distribution. No seasonal variation is recognized.

5.2.5. Age, Sex, Race, Occupation, Occurrence in Different Settings, Socioeconomic Factors, and Other Factors. *Chlamydia trachomatis*, like other genital pathogens, has been found with increased frequency among individuals who are younger, more often nonwhite, unmarried, and of lower socioeconomic status. Both males and

females with infection tend to have had more sexual experience in terms of the number of recent sexual partners. Pregnant women with *C. trachomatis* also tend to be of lower gravidity. These factors also characterize the infants who contract conjunctivitis or pneumonia. No distinctive characteristics of diseased versus nondiseased infected infants have been found. Infant conjunctivitis develops at 5–14 days of life (rarely later to 6 weeks, which may be recurrences). Infant pneumonia develops from 2 weeks to 4 months of age. *Chlamydia trachomatis* pneumonia in older children and adults is rarely seen.

5.3. Lymphogranuloma Venereum

5.3.1. Prevalence and Incidence. Reported cases of LGV have been decreasing in the last few years from 394 in 1974 to 235 in 1994 (the rates have changed from 0.2 to 0.1/100,000 population).[62] The disease is not reportable, and the recent use of molecular techniques to diagnose rectal LGV in homosexual males is not yet reflected in the reported statistics.

5.3.2. Epidemic Behavior and Contagiousness. LGV is transmitted by sexual contact, but the degree of contagiousness has not been measured. Likewise, the epidemic behavior of the disease has not been well documented. The disease was more prevalent in the United States in the first half of this century than it is at present.

5.3.3. Geographic Distribution. In the early part of this century, LGV was more common in the southeastern United States. The present geographic distribution shows a higher prevalence in some of these states (e.g., Virginia, Georgia, and Louisiana). When examined by city, the highest prevalences are in Washington, DC, Atlanta, Houston, and New York. Although some of this concentration can be attributed to better diagnosis in certain centers, it is clear that this also reflects imported disease, particularly in merchant seamen and military personnel. Regarding the source of infection overseas, the disease is more often seen in tropical climates, notably in Africa, Central America, and tropical areas of Asia.

5.3.4. Temporal Distribution. There are no data on temporal distribution.

5.3.5. Age and Sex. This disease is recognized more often in mid-adult life and more often in males than in females.

5.3.6. Race. Although a greater susceptibility of blacks was postulated in years past, such a hypothesis cannot be substantiated, and no difference in racial susceptibility is believed to exist.

5.3.7. Other Factors. The only other population group that appears to be at higher risk are sexually active homosexuals. In recent studies in large cities such as New York, San Francisco, and Seattle, infections have been found among homosexuals, particularly in men with proctitis. However asymptomatic infections are common.

5.4. Psittacosis

5.4.1. Prevalence and Incidence. Psittacosis is a relatively rare disease in the United States. In 1994, 38 cases were reported (0.2/1,000,000 population).[62] Most updated cases are associated with exposure to psittacine birds of domestic or foreign origin. In 2004, only 12 cases were reported.[63] Undoubtedly much of the decrease over recent years is due to the assumption that *C. pneumoniae* is the likely cause of any atypical pneumonia with positive chlamydial serology, except when there is a well-defined avian exposure. Cases associated with poultry processing decreased in the 1970s and then stabilized. An exception was 1984 when a particular outbreak reversed that trend.[64]

All birds entering the United States (except from Canada) are required by the U.S. Department of Agriculture to undergo a 30-day quarantine, during which time they are treated with chlortetracycline. So far, strains remain susceptible to tetracycline. It has been proposed that the quarantine period be extended to 45 days, particularly for larger psittacine birds. The current importation is about 700,000 birds per year.

5.4.2. Epidemic Behavior and Contagiousness. In 1929, epidemic disease from infected psittacine birds spread from Argentina to England, Germany, and the United States as infected psittacine birds were transported to those countries. Discovery of the cause of this disease and its treatment resulted in control of epidemic disease by the 1950s and cessation of such outbreaks. From the 1950s on there were sporadic outbreaks in poultry-processing plants. From 1979 to 1984, there were no significant outbreaks in the United States. In 1984 an outbreak occurred in turkey slaughtering plants, but there have been no outbreaks reported since then.

Human-to-human transmission can occur from a heavily infected pneumonia case, but such occurrence is extremely rare and is of no significance in the epidemiology of the disease. The important epidemiological source remains either psittacine birds or poultry flocks.

5.4.3. Geographic Distribution. There are too few cases to comment here.

5.4.4. Temporal Distribution. The distribution of cases has no distinct seasonal pattern.

5.4.5. Age, Sex, Race, and Occupation. Cases occur more often in mid-adult life and more often in males than in females, reflecting the increased exposure in these groups. There is no evidence of racial predilections. Regarding

occupation, most cases of psittacosis in recent years have occurred in owners of pet birds, employees of the commercial pet bird trade, bird fanciers, pigeon fanciers, and a variety of miscellaneous avian exposures. Since 1974, few cases have occurred in poultry producers and processors, although the potential for such occurrence still exists. Only about 10% of cases occur in persons without clear-cut exposure. Thus, the majority (93% from 1975 to 1984) of cases occur in persons exposed through vocational or avocational activities.[64]

5.4.6. Socioeconomic Factors. Socioeconomic status is not of epidemiological significance.

5.5. *Chlamydia pneumoniae* Infections

5.5.1. Prevalence and Incidence. Pneumonia incidence has been measured in a health maintenance organization in Seattle. The incidence of pneumonia averaged 12 per 1,000 per year. Fifteen percent had *C. pneumoniae* infection, for an incidence rate of 1 per 1,000 per year. This was a little less than half the incidence of *M. pneumoniae* infection. It is thought that the *C. pneumoniae* estimate is a little low as it is based on serology alone.[65] Studies in many countries show that 20–80% of adults have antibody, and in most countries that prevalence is 50% or more.[20]

5.5.2. Epidemic Behavior and Contagiousness. Epidemics have been studied in military recruits in Finland and in one adjacent civilian community.[66] Attack rates varied from 60 to 80 per 1,000 men. The case-to-case interval has averaged 30 days and ranged as high as 3 months. Transmission in all instances is slow, with epidemics lasting as long as 6 months in groups such as military and as long as 3 months for households.

5.5.3. Geographic Distribution. The original isolate was from Taiwan. The most extensive studies have been in the United States, but the prevalence of antibody in adults in most countries studied has been 50% or more. Other countries studied heavily have been Finland, Denmark, Sweden, Canada, Taiwan, and Japan.[20]

5.5.4. Temporal Distribution. The disease can occur in any season. There is some evidence of periodicity, with higher incidence rates of infection for 2 or 3 years followed by 4 or 5 years of low incidence.[65]

5.5.5. Age and Sex. Antibody is uncommon under the age of 5 years, and then age-specific prevalence increases rapidly from 5 to 20 years. Both of these characteristics are similar to the seroepidemiologic findings for *M. pneumoniae*. The prevalence then increases slowly but steadily throughout life. In Seattle, men over 60 years have a prevalence of 70%. Rates are higher in men than in women after the age of 20 years. With current serologic methods there is some loss of antibody with time (3–5 years after the first infection), so that the high rates of antibody after 70 years suggest that virtually everyone is infected at some time in their lives. Reinfections can occur.

5.5.6. Race, Occupation, Occurrence in Different Settings, and Socioeconomic Factors. There are no data that suggest significant differences by race or occupation. The significant excess in males in adult life, as well as the experience in epidemics, suggests that infection may occur more often outside the home in settings such as schools, institutions, and the military.

6. Mechanism and Routes of Transmission

6.1. Trachoma

The conjunctival epithelium of humans constitutes the reservoir of infection for trachoma. It is passed from eye to eye by direct contact. In some instances, this is aided by local practices. For example, in India, it is usual for dye to be applied around the eye, with a small stick or with the finger. This is applied to adult women first and then down to children of both sexes—from the oldest to the youngest. In many Southeast Asian countries, washcloths are applied to the face—again from eldest to youngest, and in crowded areas of poverty, usually the same cloth without a change of water. Both are ideal methods for transmission.

Extraocular infections are known to occur in trachoma endemic areas. The organism can be recovered from throat nasopharnygeal and rectal swabs.[67] It is not known what role such infections may play in transmission of the organism. They are likely to be more important in that they provide a rationale for treatment failures to occur following application of topical antibiotics, as they can be sources of reinfection.

Transmission is also potentiated by flies.[68] They will differ as to species, but in each case they are capable of carrying infected secretions from face to face, where they may be self-inoculated into the eye. Jones[46] showed by the use of fluorescein dyes that ocular discharges could be transferred from person to person in the household in a matter of minutes. He referred to the sum of these various methods of transfer of infected secretions as "ocular promiscuity" and suggested that this characterizes the epidemiological pattern of endemic trachoma in much the same manner as genital promiscuity characterizes the method of transmission for oculogenital diseases. Fly control and provision of latrines are beneficial.[68]

Two other factors play some part in the transmission of endemic trachoma in certain areas of the world—perhaps in preparing the eye for more severe infection. One is the occurrence of other bacterial conjunctivitis—often also fly

transmitted. In certain settings, seasonal epidemics of bacterial conjunctivitis precede the peak of trachoma infectivity by a few weeks. A variety of bacterial pathogens have been implicated, particularly certain *Haemophilus* and *Neisseria* species. It is surmised that the bacterial infections increase both conjunctival and corneal inflammation, and thus amplify their subsequent response to trachomatous infection. Anyone who has been in such areas at the height of the fly season can attest to the intensity of fly infestation. Flies cover the faces of children, particularly around the eyes, where there is conjunctival discharge.

A second set of factors less well understood are the environmental factors that characterize many of the foci of greatest prevalence. These are dust and sunlight—both irritants to conjunctiva and cornea and both hypothesized to aid *C. trachomatis* in its damaging effects. It is difficult to measure these influences, but trachoma remains most prevalent and most damaging in those locations where these factors predominate.[46]

6.2. Oculogenital Diseases

The columnar cells of the adult male urethra and female endocervix are felt to be the reservoirs of infection. Asymptomatic infection seems to be the rule: depending on the type of clinic >50% of infected men and women with positive urethral or cervical cultures have no complaints or discharge. The organism is transmitted via sexual contact.

In women, *C. trachomatis* is responsible for a proportion of acute and chronic cervicitis on examination, but how much is difficult to say, since it is often accompanied by other pathogens. Urethral syndrome or acute urethritis may be a result of simultaneous infection, spread from infected cervical secretions. The organism is responsible for at least 20–40% of cases of salpingitis, presumably via ascending infection. Postpartum endometritis, thought to be at least partly due to *C. trachomatis*, also occurs via direct extension.

In males, the major illnesses (urethritis and epididymitis) are due to direct extension of infection from the primary urethral focus. Hematogenous dissemination has never been documented in either sex. Adult inclusion conjunctivitis is felt to be transmitted via hand-to-eye contact from infected genital foci.

The only documented mode of transmission of infection to infants is vertically by passage through an infected cervix. No hard evidence for transplacental or postnatal infection exists.

6.3. Lymphogranuloma Venereum

The reservoir of infection is the cervix of the asymptomatic female and, to a lesser extent, for the male homosexual, the rectal mucosa of the asymptomatic male. It is spread exclusively by sexual contact. In the male, primary lesions appear on the penis, usually on the glans (occasionally in the urethra), or the wall of the rectum, and in the female on the labia or on the wall of the vagina. Such lesions are usually painless and may be hidden, adding to the risk of transmission. Extragenital sites for primary lesions include hands (especially fingers) and tongue. Although secondary and tertiary lesions are the ones most easily diagnosed and are the stages of disease that result in damaging sequelae, it is the painless primary lesions that are the source for sexual transmission.

6.4. Psittacosis

The predominant sources for human disease are infected avian species, all of which are potential reservoirs. Meyer[69] listed 130 species known to be such sources in 1967, and the list could no doubt be lengthened. The risk of exposure is greatly increased in those occupations in which handling birds or their carcasses is common. The most common avian sources are parrots and parakeets, usually resulting in sporadic cases from pet birds. In recent years, about two-thirds were from psittacine species. Pigeons are another source, as are poultry, particularly turkeys. Wild birds have infrequently been documented as a source. Domestic and other lower mammals are infected with *C. psittaci* strains, but they rarely cause human disease. An exception may be the chalmydiae associated with ovine abortion. These appear to specifically threaten the health of pregnant women. In the overwhelming majority of cases, psittacosis occurs in persons in whom bird processing or handling is a vocation or avocation. Casual exposure is extremely rare.

Transmission between humans can take place, and on rare occasions such transmission has been documented. It usually has resulted from close exposure to extremely sick or fatal cases.

The route of infection is airborne, and the primary site of infection is the lower respiratory tract. The involvement is in the alveoli, with very little evidence of bronchial or upper respiratory tract infection.

6.5. *Chlamydia pneumoniae* Infections

There is no evidence for other than human-to-human transmission of *C. pneumoniae* infection. Clearly epidemics occur, as described in the pneumonia epidemics in military trainees in Finland.[66] Because the organism was originally thought to be a member of the *C. psittaci* species and because it can infect experimental animals, an extensive search for antibody in serum of domestic pets was made and has yielded

no evidence for an animal reservoir. In addition, there is no epidemiological evidence to suggest an unusual frequency of animal contact in cases.[20] *Chlamydia pneumoniae*-like strains have been recovered from horses, koalas, and frogs. It is not known whether these strains have anything to do with human disease. Genomic sequences demonstrate that these strains are quite similar, but not identical to *C. pneumoniae* isolates from humans.

7. Pathogenesis and Immunity

7.1. Trachoma

In the early stages of disease, trachoma appears as a follicular conjunctivitis with hyperemia, edema, and distortion of the vascular pattern of the conjunctiva. This is accompanied by papillary hypertrophy of the conjunctiva. The follicles are, in fact, lymphoid germinal centers. In these initial stages, there is involvement of both palpebral and bulbar conjunctivae. Such conjunctival follicles can occur as a response to a number of stimuli, both infectious and toxic. The infant conjunctiva cannot so respond for a number of weeks, and thus chlamydial ocular infection of the newborn (inclusion conjunctivitis) is afollicular, even though the strains are very similar (e.g., as shown by the disease produced in the experimental monkey eye). The chronic follicular conjunctivitis of trachoma heals by scarring. These scars lead to distortion of the lids. This, in turn, results in inability of the lids to close completely, allowing dust particles to damage the cornea. Similarly, the lid distortion results in trichiasis, or inversion of lashes, which adds to the trauma to the cornea. Both these sequelae of trachoma contribute to blindness.[46] The incubation period is not known. In volunteer studies and experimental studies in the nonhuman primate it is 5–12 days.

Acute corneal involvement includes follicles, which often ring the limbus and, after rupture, lead to corneal defects termed Herbert's pits. Punctate erosions of the cornea occur (epithelial keratitis). Inflammatory infiltrates of the cornea may be seen. A characteristic feature of trachoma is the vascularization of the cornea, with swelling of the corneal–scleral border, which constitutes pannus. This is an important component of trachoma diagnosis.

Collectively, these features of trachoma constitute the acute and chronic conjunctivitis and their blinding sequelae. The occurrence of these features in all populations increases with age, although the age at which they occur will depend on the intensity of disease in each sample.

All these changes have been reproduced experimentally in the monkey, but never with the initial infection, which produces only acute follicular conjunctivitis leading to minimal scarring. Pannus is only a result of reinfection (exogenous reinfection or endogenous relapse).[20] Whether this is true for human disease has never been substantiated. If it is, then the disease that is characterized as trachoma (with pannus) is never a primary infection. Nevertheless, it is clear in human disease that the serious sequelae that lead to blindness occur only as a result of many reinfections, or persistent infection and high infectious load.[70,71]

It has been postulated that hypersensitivity reaction to specific *Chlamydia* heat shock proteins may be responsible for the inflammation seen on reinfection.[72]

If reinfection is a necessary factor for severe trachoma, what is the role of immunity? In the experimental animal, after infection, for a short period, there is immunity to reinfection with homologous strains (but no immunity to heterologous rechallenge). In the few studies that were made with what are now recognized as relatively crude and antigenically weak vaccines, there was transient immunity to rechallenge. When this immunity waned, not only were the experimental animals susceptible to reinfection, but also the severity of disease produced by such rechallenge was enhanced (a phenomenon similar to that seen in other early and crude experimental vaccines, such as those for *M. pneumoniae* and respiratory syncytial virus). Whether such a period of hyperreactivity exists in natural human infection has not been determined, but immunity in nature is fleeting. Infection in humans and in experimental animals produces both local and circulatory immune responses, but these antibodies do not appear to be protective. One epidemiological fact continues to kindle interest in the prospect of producing an effective vaccine for trachoma—the observation that in all endemic situations studied, active trachoma decreases with age. Although the age at which this occurs will vary with the intensity of disease in the population, eventually the older segment of the population no longer has active disease, suggesting that immunity eventually prevails. This occurs at an early enough age in some populations that one cannot attribute it to decrease in ocular promiscuity alone. It is the aim of proponents of vaccine development to create a vaccine that would mimic the natural occurrence.

7.2. Oculogenital Diseases

There are several useful unifying principles in regard to the oculogenital infections caused by *C. trachomatis*: (1) the organism infects superficial columnar epithelial cells in various sites (endocervix, urethra, epididymis, endometrium, salpinx, conjunctiva, nasopharynx, lower respiratory tract) and appears to have little propensity for invasion of or destruction of deeper tissue. (2) The clinical features of

chlamydial infection seem to be produced by the host inflammatory response, rather than by the inherent destructiveness of the organism. (3) *Chlamydia trachomatis* infections may be present and asymptomatic ("latent" or "silent" infection) for prolonged periods of time. For example, infants have been documented to shed organisms from conjunctivae for up to 2 years postdelivery. Similarly, women with cervical infection have been culture positive for over 15 months. (4) The stimuli that convert latent infection to chronic disease, or that determine symptomatic versus asymptomatic primary infection, are unknown. (5) At least in some cases (pregnant women), infection can occur, recur, and persist even in the presence of serum antibody. Cell-mediated immune responses (as measured by lymphocyte blastogenic response) appear to be associated with clearing infection, although the evidence is far from clear.

The incubation periods for oculogenital syndromes vary with the syndrome. Urethritis appears to have an incubation period of 5–14 days. This is longer than the incubation period for gonococcal urethritis, which is 2–7 days. When they occur as a result of concurrent exposure, the chlamydial urethritis usually occurs later, which accounted for the entity known as postgonococcal urethritis that occurred in some cases of gonococcal urethritis treated with penicillin only. The incubation period for cervicitis is probably the same as urethritis, but because of the slow and infrequent development of symptoms, is harder to define. The incubation period for chlamydial salpingitis is longer usually following cervical infection by 2–3 weeks. The incubation period for conjunctivitis of infants, children, or adults is 5–12 days. In the case of ophthalmia neonatorum, chlamydial ophthalmia most often has onset in the second week of life as opposed to gonococcal conjunctivitis which occurs characteristically in the first week. Chlamydial pneumonia of infancy has onset between 3 and 12 weeks of age. From prospective studies it appears to follow the earliest recognition of posterior nasopharyngeal infection by a week or so. Rectal shedding is rarely discovered before the second month of life.

In all illnesses so far studied, the surface chlamydial infection appears to produce an early polymorphonuclear inflammatory response at the surface and a later subepithelial infiltration of lymphocytes, plasma cells, monocytes, and eosinophils. Inclusions have been seen in cervix, conjunctiva, salpinx, and infant airways. Although the antigen load, in terms of inclusions seen, does not appear great, the immune response appears to be profound. In infants with chlamydial pneumonitis, for example, large interstitial and peribronchiolar lymphoid infiltrates and organizing nodules with germinal centers can be seen; these are the "infiltrates" seen on chest radiography.

The preliminary evidence available indicates that chlamydial infections may produce chronic sequelae. The observations are not unexpected, since the intense inflammatory response produced by chlamydial infection can lead to tissue destruction, fibrosis, and scarring. Data from Sweden suggest that chlamydial salpingitis has a higher postinfectious incidence of involuntary infertility and ectopic pregnancy than does gonorrheal disease. Distal tubal obstruction has been reproduced in the nonhuman primate by repeated salpingeal infection.[73] Furthermore, obliterative bronchiolitis has been observed in the lung biopsy specimen taken from an infant recovering from chlamydial pneumonitis.

7.3. Lymphogranuloma Venereum

The primary lesion of LGV is usually painless and may often be missed. The incubation period is variable and has been described as 3–30 days. The primary lesion may be a papule, an ulcer or erosion, a vesicle, or a urethritis. Rarely, the site may be extragenital. Pathologically, there is nothing specific about these lesions. Proctitis commonly accompanies primary anorectal infection.

In most instances, it is the secondary lesions that bring the disease to medical attention. These are involvement of lymph nodes, producing the characteristic bubo. The bubonic form occurs in males more often than females (9:1), in part due to differences in lymphatic drainage patterns. Both inguinal and femoral node chains can be involved (when both are involved, the characteristic groove sign results—a groove between the two chains). Systemic signs (fever, myalgia) may occur at this time, but leukocytosis is rare. Large mononuclear cells proliferate within the nodes and foci become necrotic. These foci may proceed to suppuration and rupture of the nodes, developing draining fistulas. Alternatively, the nodes may spontaneously harden and then heal. In approximately 5% of cases, a chronic lymphadenopathy may occur, which may persist for many years.

What are sometimes referred to as tertiary lesions of LGV is analogous to the sequelae of trachoma in that they are the result of scarring. They include strictures of the rectum and vagina and fistulas that may be rectovaginal or may involve the urethra. These lesions are characterized by destruction of mucosal epithelium with scarring and plasma cell infiltration. Extragenital involvement may rarely involve the conjunctivae, the skin (e.g., fingers), and the oral mucosa—each with respective lymph-node involvement. Even less frequent are syndromes of meningitis, salpingitis, arthritis, and pneumonitis.

LGV elicits a marked host response. Nonspecifically, this is reflected in a hypergammaglobulinemia that may

result in a reversal of albumin/globulin ratio. Specifically, broad and intense response can be measured with chlamydial antigens. For that reason, CF antibodies are uniformly elevated, and this generally insensitive test is useful in this disease, utilizing *C. psittaci* antigen. Using specific MIF antigens, a brisk response is noted, which is characteristically broad (responding to many *C. trachomatis* antigens). Although the immunological response can be readily measured, less is known concerning immunity in this disease. Reinfection has not been reported, but chronic disease can occur. Cellular immunity is poorly studied in this infection, but skin-test reactivity and the prominent tissue damage in lymph nodes suggest that such immunity is operative.

7.4. Psittacosis

The respiratory tract is the organ of entry and initial multiplication. The incubation period is between 6 and 15 days (rarely longer). In the experimental monkey, initial respiratory infection is followed by regional lmphadenopathy (tracheobronchial lymph nodes). Bacteremia occurs, followed by involvement of the reticuloendothelial system with early hepatic infection and persistent splenic infection. Monocytic and polymorphonuclear infiltration of the tracheal wall marks the initial respiratory lesions, but in human specimens from fatal cases, bronchiolar involvement is not found. The characteristic lesion of psittacosis is a serofibrinous alveolar exudate that results in focal or lobar consolidation. The alveoli contain both fibrinous red cells and polymorphonuclear cells. In later stages, alveolar septal-cell hyperplasia with phagocytic infiltration occurs.

The splenomegaly results from lymphoid infiltration with an increase in mononuclear phagocytic cells. Focal necrosis and granulomata may occur in the liver. CNS involvement occurs infrequently and is characterized by congestion and edema of the brain or spinal cord.

Host response to *C. psittaci* infection can be measured in a number of ways, and it is clear that both circulating and cellular immune responses occur. On the other hand, there is no evidence for lasting immunity in either animal or man following this infection. Reinfection is well documented, as is persistent infection under some circumstances.

7.5. *Chlamydia pneumoniae* Infections

The pathogenesis of *C. pneumoniae* infections is not understood. There have been no lung biopsies, deaths, and there is no truly useful experimental model. Our knowledge is limited to clinical observation and the analogy to *M. pneumoniae* pneumonia. The incubation period for *C. pneumoniae* infection is not known. The case-to-case interval averages 30 days. Reinfection has been documented, even in the same epidemic.[66]

8. Patterns of Host Response

8.1. Trachoma

8.1.1. Clinical Features. The initial response to infection with *C. trachomatis* is a conjunctivitis. This involves both palpebral and bulbar conjunctivae, and in both areas, congestion and edema are the first responses, with papillary hypertrophy prominent in the palpebral conjunctiva. This is followed by follicle formation (except for infants less than 6 weeks of age, in whom follicle formation is not possible). Follicles can also be found on the bulbar conjunctiva. Those that form on the limbal border are most characteristic. After rupture, they leave shallow scars, which are termed Herbert's pits. In hyperendemic trachoma, severe disease is more characteristic of the upper tarsus. The conjunctivitis produces an ocular discharge that is initially serous, but with congestion can become serosanguinous. Since trachomatous conjunctivitis is usually a subacute process, necrosis is minimal, and therefore pure trachomatous disease is not particularly purulent (with the exception of infant conjunctivitis, which has either purulent or mucopurulent discharge). Unfortunately, in hyperendemic areas, bacterial infection often accompanies the initial disease, and thus the discharge is often very purulent. In trachoma, corneal involvement is usually present, producing punctate erosions of the epithelium with cellular infiltrates of the central corneal epithelium and anterior stroma (keratitis). Particularly characteristic of hyperendemic trachoma is a neovascularization (pannus) at the superior limbus. This sign is an important component in the diagnosis of trachoma.

In either classic trachoma or adult inclusion conjunctivitis, the most damaging aspects of the disease are the sequelae of reinfection. Follicles of the palpebral conjunctiva scar, which results in distortion of the lids. This may result in trichiasis (inturning of the lashes to scrape the cornea) and entropion (distortion of the lid margins secondary to scarring, resulting in failure of the lids to close, in turn resulting in disturbance of the protective cleansing action of the lids and therefore further corneal scarring, particularly in dusty environments). These changes, along with the recurrent keratitis, in company with multiple reinfections and bacterial superinfections result in loss of visual acuity and may result in blindness.

All these changes are seen, but to a lesser degree, in adult inclusion conjunctivitis. The difference in sequelae between trachoma and inclusion conjunctivitis is probably related more to the greater number of reinfections in hyperendemic trachoma and the adverse physical environment (including the effect of fly-borne bacterial conjunctivitides) than it is to the biological difference between trachomatous and oculogenital chlamydial strains. When reinfection has been studied in nontrachomatous environments [e.g., the studies of Mordhorst et al.[74] in Denmark], each of the sequelae of trachoma has been recognized, but in lesser severity.

8.1.2. Diagnosis. Epidemiologically, trachoma is recognized in restricted areas of the world (see Section 5.1.3) and within those areas can most often be found in populations with poor hygiene and under conditions that favor "ocular promiscuity" (often also favoring fly-borne bacterial conjunctivitis). Other isolated population segments in nonendemic countries may have endemic trachoma.

According to WHO[27] definitions, the clinical diagnosis of trachoma can be made if two of the following signs are present: (1) lymphoid follicles on the upper tarsal conjunctiva; (2) typical conjunctival scarring; (3) vascular pannus; and (4) limbal follicles or their sequelae—Herbert's pits. Scales of severity and activity have been used to classify trachoma. The most commonly used staging is called the MacCallan classification, which progresses from immature follicles (I) to mature follicular disease (II), active follicles with scarring (III), and scars and other sequelae without activity (IV).

For epidemiological survey purposes, Dawson et al.[75] have devised a scale of intensity based on three gradations of follicle activity and papillary hypertrophy. For the potentially disabling irreversible lesions, simple three-grade scores for congenital and corneal scarring and for trichiasis or entropion or both have also been devised. This method leads to more reproducible and epidemiologically valid results in surveys.

Other syndromes can cause follicular conjunctivitis, including viral and bacterial infections, toxic follicular conjunctivitis, vernal catarrh and atopic conjunctivitis (allergic in origin), and a variety of rarer forms of chronic follicular conjunctivitis of unknown etiology.

The laboratory diagnosis of *C. trachomatis* infection in trachoma is best made by NAATs. In the past it depended on the recovery of *C. trachomatis* from conjunctival scrapings or the demonstration of characteristic inclusions in the cytoplasm of conjunctival epithelial cells obtained from such scrapings. These may most easily be recognized with Giemsa stain, but DFA methodology is more sensitive.

An advantage of the Giemsa stain in cytological diagnosis is the recognition of the characteristic inflammatory response. Whereas viral conjunctivitis usually elicits a lymphocytic response, and vernal catarrh and atopic conjunctivitis can be recognized by the presence of eosinophils or granules, the characteristic picture in trachoma or inclusion conjunctivitis is a mixture of polymorphonuclear leukocytes, immature lymphoid cells, and plasma cells. Also, Leber cells (giant macrophages containing ingested material) are often seen. This inflammatory response, while not in itself diagnostic, may aid in the cytological diagnosis of trachoma.

Although antibodies to *C. trachomatis* may invariably be recognized in the sera of trachomatous patients by MIF methods, it is unusual to detect an antibody rise, since the disease is most often recognized in its chronic state and therefore reflects only past infection with a *C. trachomatis* strain.

8.2. Oculogenital Diseases

8.2.1. Clinical Features (see Table 1)

8.2.1.1. Adult Inclusion Conjunctivitis. Adult inclusion conjunctivitis in both the male and the female is an acute follicular conjunctivitis with edema, mucopurulent exudate, and erythema. Inclusions can be found on scrapings of either the tarsal plate or the lower palpebral conjunctiva, and chalmydiae can be cultured from either site. Infection is thought to occur from infected genital tract secretions via hand-to-eye contact. The illness is self-limited, although treatment will shorten the clinical course. Progression to trachoma has not been documented, but pannus and conjunctival scarring may result from reinfection.

8.2.1.2. Cervicitis. Chlamydial infection of the endocervix is often associated with a purulent endocervical discharge, congestion, and inflammation. The essential finding is the presence of mucopus (discoloration) on a cervical swab. Initially the criterion of 10 or more polymorphonuclear leukocytes per microscopic field was also used, but the difficulties of standardizing the smears made this unreproducible. A more reliable criterion, in addition to mucopurulent exudate, was bleeding induced by swabbing the endocervical mucosa (friability of the mucosa).[76] More than 50% of women with chlamydial cervicitis are asymptomatic, but many may have an abnormal cervical appearance. The difficulty in diagnosing chlamydial cervicitis is that the findings present are often the same as those found with cervicitis of other etiologies. Furthermore, the cervix is often infected with several other organisms simultaneously.

Under the proper stimulus (as yet undefined), chlamydial cervicitis may progress to salpingitis, or to postpartum endometritis in pregnant women, and it may be associated

with urethral syndrome. Endometrial infection has been shown to follow cervical infection in more than one-third of cases.[77] An inflammation of Bartholin's gland may also be caused by *C. trachomatis*. Without progression to other sites, chlamydial cervical infection may remain active but silent, or it may be cleared by the host either spontaneously or with treatment.

8.2.1.3. Salpingitis. Chlamydial pelvic inflammatory disease is similar in presentation to that caused by other organisms, with the exception that it tends to be of more gradual than acute onset and to exhibit low-grade fever, and often has elevation of the erythrocyte sedimentation rate (>30 mm/h). Like gonorrheal illness, it tends to occur in younger, more sexually active women, and as a first rather than recurrent episode. Major signs and symptoms include fever, lower abdominal pain, and adnexal and uterine tenderness on pelvic examination. The diagnosis of chlamydial infection has proven difficult, however, since the organism may reside solely in the salpinx. Thus, cervical or cul-de-sac cultures may yield gonorrhea or anaerobes, yielding a false diagnosis. The clearest results have been obtained in Sweden using laparoscopy with tubal biopsy,[54] but this is neither a universally available nor an accepted technique for mild illness. The contribution of chlamydial salpingitis to involuntary infertility and to ectopic pregnancy in the United States is not known, although both have increased in parallel with increase in the incidence of salpingitis. Assuming 1 million cases of salpingitis per year in the United States and 15–20% resulting in bilateral tubal occlusion, it has been estimated that 150,000–200,000 women per year become infertile as a result of salpingitis—at least one quarter of which can be attributed to chlamydial infection.[78]

An uncommon complication of salpingitis is perihepatitis (Fitz-Hugh–Curtis syndrome), assumed to be caused by peritoneal spread of the infection. Studies have implicated *C. trachomatis* as etiological agent in a proportion of these cases.

8.2.1.4. Female Urethral Syndrome. This syndrome is the equivalent in women of NGU in men. It is predominantly manifest as frequency dysuria, with sterile pyuria on microscopic and cultural examination, in young, sexually active women. In this specifically defined group, one study revealed a 62% (10 of 16) prevalence of *C. trachomatis* as the etiological agent.[79] Other studies show a lesser contribution. Often, there is a history of a new sexual partner in the month prior to symptoms. Untreated, the urethral syndrome will resolve with time. Long-term sequelae are as yet undetermined.

8.2.1.5. Postpartum Endometritis. Chlamydia trachomatis cervical infection in pregnant women has been associated with the development of postpartum endometritis, with onset more than 48 h after delivery in approximately one-third of cases.[56] The disease is not different clinically from that due to other organisms.

8.2.1.6. Male Urethritis. Chlamydial infection of the adult male urethra is the most common single cause (30–50%) of "nongonococcal urethritis" (NGU) or "postgonococcal urethritis." This is a syndrome of dysuria with or without discharge, with pus cells on Gram smear of an endourethral swab, in which *Neisseria gonorrhoeae* can be neither seen nor cultured. The disease occurs in young, sexually active males and is at least equal in prevalence to gonococcal urethritis in the United States. Untreated, it may lead to epididymitis or resolve spontaneously. Treatment shortens the clinical course.[57]

8.2.1.7. Epididymitis. Chlamydia trachomatis is the most common cause of "idiopathic" acute epididymitis in sexually active man under 35 years of age with no underlying genitourinary pathology.[58] The prevalence of the organism in the urethra, semen, and epididymis of these men far exceeds that of *N. gonorrhoeae*, coliforms, or *Pseudomonas* species. Patients with chlamydial epididymitis tend to have a more chronic course, with more induration of the epididymis, than those with other etiological diagnoses. The illness will resolve spontaneously, although treatment hastens recovery. The incidence of infertility following chlamydial epididymitis is unknown. However, chlamydial epididymitis is often associated with oligozoospermia.

8.2.1.8. Arthritis (Reiter's Syndrome). One of the most serious complications of NGU is Reiter's syndrome. Symptoms appear 1–4 weeks after onset of urethritis. The arthritis is usually asymmetric, involving large joints of the lower extremities or sacroiliac joints. In most patients, arthritis may be the only manifestation. Achilles tendon and plantar fasciae may also be affected. This condition has been termed sexually acquired reactive arthritis (SARA). The full expression (termed Reiter's syndrome) may additionally involve eyes, skin, and mucous membranes. Ocular involvement ranges from a transient mild conjunctivitis to severe uveitis. Skin lesions may involve the penis (balanitis) and a keratoderma of the palms and soles. The mucous membrane lesions are small ulcers of the palate, tongue, and oral mucosa. Symptoms eventually will cease, even without treatment, but recurrences are common.[59] Other infections (not sexually transmitted), such as *Shigella* and *Salmonella*, can also cause Reiter's syndrome.

Chlamydia trachomatis has been recovered from the urethra of at least one-third of cases of SARA, but serological evidence suggests that one-half to two-thirds are a result of this infection. Antibody responses are much higher than are usually seen in NGU, suggesting that a brisk immunological response is a part of the condition. A genetic predisposition to the development of SARA is marked by

the presence of histocompatibility determinant HLA-B27 in two-thirds of cases. Additional evidence for *Chlamydia* etiology comes from examining synovium or synovial fluid cells with fluorescein-labeled monoclonal antibody to *C. trachomatis* and demonstrating elementary bodies in the joint.[80]

8.2.1.9. Infant Inclusion Conjunctivitis. Inclusion conjunctivitis of the newborn is by far the most common cause of neonatal conjunctivitis in the United States and is estimated to be 10 times as prevalent as gonococcal ophthalmia neonatorum.[81] Approximately 25% of infants born to cervix-positive mothers will develop some degree of chlamydial conjunctivitis, involving one or both eyes. The illness most commonly has an incubation period of 1–3 weeks. Infants then present with varying degrees of conjunctival erythema, edema, and mucopurulent discharge. Physical examination shows a *nonfollicular* conjunctivitis (as distinct from the conjunctival disease of older children and adults) with diffuse erythema of the conjunctivae. Fever and systemic findings are absent. Untreated, the disease course is quite variable. Generally, the conjunctivitis remits within 3–4 weeks, although asymptomatic shedding may persist indefinitely. Occasionally, re-exacerbations may occur for unknown reasons. Chronic sequelae do occur; in one study, micropannus was observed in a small percentage of infants 1 year following infection.

8.2.1.10. Infant Pneumonia. Chlamydia trachomatis pulmonary infection has been estimated to cause 20–30% of all hospitalized pneumonia in infants less than 6 months old.[81] Approximately 10–20% of infants born to cervix-positive mothers will develop pulmonary disease. These infants typically present between 3 and 12 weeks of age, although younger patients have been seen. They have a history of chronic congestion and of a chronic staccato, machine-gun-like cough.[82] They are usually afebrile, with minimal malaise, but may have a history of poor growth and weight gain. On examination, they tend to be congested and tachypneic and to have diffuse rales on physical examination. Conjunctivitis is sometimes present. Laboratory examination characteristically reveals eosinophilia (>300–400/mm^3), hyperglobulinemia (especially IgM), and elevated antichlamydial antibody titers. Chest radiographs show hyperexpanded lungs with variable degrees and patterns of infiltrates. The organism itself may be cultured from nasopharynx, tracheal aspirate material, or lung biopsy, if obtained. Although pneumonia is best studied, other forms of lower respiratory tract disease can occur (e.g., bronchiolitis).

Untreated, the illness tends to be chronic, with multiple exacerbations and remissions, but with gradual recovery over weeks to months. Although placebo-controlled therapy trials have not been performed, appropriate antibiotic therapy does eliminate shedding of the organism and probably shortens the course of the illness.

8.2.2. Diagnosis. The major epidemiological features of chlamydial genital diseases are that they occur in predominantly younger, more sexually active adults who are more often single, of lower socioeconomic status, and more often nonwhite. This is the pattern expected in a sexually transmitted disease. Infant disease may be expected in a proportion of those born to infected women.

In all of the syndromes listed below, NAATs would be considered the diagnostic tests of choice.

8.2.2.1. Adult Inclusion Conjunctivitis. The diagnosis is suspect if a follicular conjunctivitis is present. Wright- or Giemsa-stained conjunctival scrapings will reveal inclusion-containing epithelial cells if done carefully and examined in an experienced laboratory. Similar diseases are caused by bacteria (including *Neisseria meningitidis* and *N. gonorrhoeae*) and viruses (especially adenovirus). Bacterial conjunctivitis is usually purulent, without follicles.

8.2.2.2. Cervicitis. Clinical features include mucopurulent discharge, ectopy (redness), with edema, friability, and congestion. Laboratory diagnosis is difficult, at best, since a positive chlamydial endocervical swab or paired serological fourfold titer change is required. Wright- or Giemsa-stained cervical scrapings examined for inclusions are not generally helpful. Other organisms associated with a similar picture include *N. gonorrhoeae*, *Mycoplasma hominis*, herpes simplex, and possibly *Trichomonas vaginalis* and *Ureaplasma urealyticum*. The presence of mucopurulent exudate is most frequently associated with chlamydial or gonococcal infection.

8.2.2.3. Salpingitis. Epidemiological and clinical features are discussed in Section 8.2.1.c. Laboratory diagnosis is most definitively made with tubal culture of biopsy under laparoscopy. Endometrial culture has the best correlation with tubal infection. Other sites in which chalmydiae may be cultured with less reliability include cervix and pelvic cul-de-sac. Serologically, a fourfold or greater rise in antichlamydial antibody is usually demonstrable.

Other agents associated with salpingitis include *N. gonorrhoeae*, gram-negative enteric organisms such as *Escherichia coli*, and anaerobic flora.

8.2.2.4. Female Urethral Syndrome. Diagnosis of chlamydial etiology is, first, by exclusion of other bacterial etiology on urine and urethral culture and, second, by the culturing of *C. trachomatis* from the urethra or cervix. Other etiological agents include coliforms, staphylococci, and possibly ureaplasmas.

8.2.2.5. Postpartum Endometritis. Information on this syndrome is still limited. However, diagnosis may be

established by obtaining *C. trachomatis* from endometrial curetting, aspirates, or swabs. The diagnosis is suspect if *C. trachomatis* is obtained on cervical culture. A serological change also occurs in this infection. Endometritis is also caused by *N. gonorrhoeae*, coliforms, and anaerobes.

8.2.2.6. Male Urethritis. Diagnosis is similar to that of urethral syndrome, except that in this case gonococcal infection must be ruled out. Other known causes of NGU include *U. urealyticum* and *Mycoplasma genitalium*.[83] In 20–30% of cases, no agent is isolated or serological conversion demonstrated.

8.2.2.7. Epididymitis. Diagnosis is made by exclusion of bacterial agents in urine, urethra, or epididymis (coliforms, *Pseudomonas*, *N. gonorrhoeae*) and by culture of *C. trachomatis* from urethra or epididymal aspirate, or both, or demonstration of seroconversion.

8.2.2.8. Arthritis (Reiter's Syndrome). In either SARA or Reiter's syndrome, the diagnosis is made by recovery of *C. trachomatis* from the urethra of a clinically compatible case or the demonstration of a fourfold rise in specific antibody, or both. Alternatively, the presence of IgM *C. trachomatis* antibody, or the presence of high titers of IgG antibody, is presumptive evidence of chlamydial involvement.

8.2.2.9. Infant Inclusion Conjunctivitis. NAATs are the tests of choice, but conjunctival scraping (stained by Giemsa or FA methods) or culture for *C. trachomatis* are also useful procedures. Gonococcal ophthalmia neonatorum and other bacterial agents may be excluded by culture and Gram smear. Chemical conjunctivitis (silver nitrate) rarely presents as late as chlamydial disease.

8.2.2.10. Infant Pneumonia. Diagnosis of chlamydial disease is made with a compatible clinical history and physical examination, eosinophilia, hyperinflation, and hyperglobulinemia, coupled with a positive tracheal aspirate, nasopharyngeal, or lung biopsy culture. Serological diagnosis using paired sera may be accomplished retrospectively in patients on antibiotics who are culture negative.

Since this disease occurs early in infancy, active infection may be suspected with a high titer of specific IgG antibody (particularly if it is simultaneously compared with maternal titer) or by a high titer of specific IgM antibody ($>$1:32 by MIF).[84]

Agents that cause similar pictures are *Bordetella pertussis* and *Bordetella parapertussis*, cytomegalovirus, respiratory syncytial virus, and adenovirus. Recent evidence has also suggested *Pneumocystis carinii* as an etiological agent.

Another potential diagnostic problem is differentiation from lymphocytic interstitial pneumonia (LIP) associated with human immunodeficiency virus (HIV) and Epstein–Barr virus (EBV) infections. Usually they do not occur at the same age. *Chlamydia trachomatis* pneumonia is rare after 4 months of age and LIP is rare before 6 months of age, characteristically occurring in the second year of life. Tachypnea, rather than repetitive cough, is characteristic of LIP and the radiographic pattern is more diffuse in LIP. Although both may have hyperglobulinemia, eosinophilia is not characteristic of LIP.

8.3. Lymphogranuloma Venereum

8.3.1. Clinical Features. The primary lesion of LGV may develop on the penis, labia, vagina, or cervix, or within the urethra, anus, or rectum, often resulting in proctitis. Rarely, it may be extragenital (e.g., fingers or tongue). Since it is usually painless and may be papular, ulcerative, or bullous, it rarely will cause the patient to seek medical attention, and is usually not recognized historically. The secondary lesion of LGV consists of lymphadenopathy, which may follow the primary lesion in 1–6 weeks. With genital involvement, either inguinal or femoral lymph-node chains may be involved, starting with a single node, but progressing to involve the whole chain. Abscesses form within the nodes, which expand to necrotic foci that may become fluctuant, spontaneously rupture, and leave chronic draining fistulas. These necrotic abscessed nodes are the buboes that characterize this disease—and are most commonly the initial recognition of it. The fact that a far greater number of males than females are recognized to have buboes (up to 10-fold in some reports) probably reflects the anatomical differences in lymphatic drainage from the various sites. The vaginal and cervical infections often drain to retroperitoneal chains, as do the anorectal primaries, which may produce manifestations more often confused with appendicitis or inguinal hernia. In the bubo stage, fever, myalgia, and headache may reflect systemic response to the lymphatic infection, and leukocytosis may result. Buboes may spontaneously subside and should not be surgically incised or drained, but may be aspirated. Rarely, chronic lymphadenopathy may result from this disease. The tertiary stage of disease consists of chronic inflammation and scarring, which may result in stricture and fistulas. These changes may follow acute LGV by 5–10 years. Urethral strictures may occur, as may penile fistulas. Anal strictures and rectovaginal fistulas are more often the sequelae in females (perhaps because of the more silent involvement of retroperitoneal lymph nodes). The current increase in the incidence of anorectal LGV in male homosexuals in the United States may be expected to result in increased recognition of resultant anal strictures due to tertiary LGV in the future.

In 2003, an outbreak of rectal LGV was reported in The Netherlands. Similar cases were soon identified in other countries in Western Europe and North America. These infections were detected through the use of nucleic acid amplification procedures. Sequencing of the chlamydial genes found that the cases in The Netherlands were due to a previously unrecognized genovariant of L2.[85]

There has been considerable concern about the sudden appearance of rectal LGV in these communities because the disease is quite invasive, ulcerative, and theoretically it could result in an enhanced HIV transmission or acquisition.

It has been questioned that whether there is, in fact, a new LGV outbreak, with a new strain. The likelihood that LGV is actually endemic at low levels within these communities has been raised.[86] This conjecture is based on the fact that the regularly used diagnostic procedures for *C. trachomatis* infection of the genital tract will not differentiate between LGV and infections with the trachoma biovar. Only through the use of more specific methods to identify genomic variations were these infections identified as LGV. However, the clinical condition of LGV proctitis has been recognized for decades, what appears to be a unique genovar of L2 was found to be a common type seen among isolates from rectal specimens from men with proctitis in San Francisco in the 1980s.[87]

Much rarer clinical manifestations reflect unusual extragenital sites of primary infection, such as axillary buboes with finger sites or submaxillary buboes for oral lesions. Other rare reported manifestations of LGV include meningitis, arthritis, pneumonitis, and conjunctivitis.

8.3.2. Diagnosis. Epidemiologically, LGV is found in sexually active persons, particularly those with past histories of other STDs. At one time, it was prevalent in blacks in the southern United States, but this is probably no longer true. Since the disease is more prevalent in African and Southeast Asian countries, another epidemiological feature of the occurrence in the United States has been overseas contact, such as is found in port cities or in returning military personnel who have served in these areas. In recent years, however, the disease has been found more frequently in the more sexually active male homosexual population in the United States. This segment probably represents the most frequent source of infection in the United States today.

Clinically, the primary lesion rarely comes to medical attention, and the most commonly recognized stage is that of the bubo. They are most often recognized in males, and then by inguinal or femoral node chain involvement. If both chains are involved, the "groove sign" is seen, the groove being formed between enlarged nodes of both chains. Although highly characteristic of this disease, it is seen in only 15–20% of cases.

The differential diagnosis of LGV includes syphilis (although characteristically the nodes are hard and separate in that disease), granuloma inguinale, and chancroid. Other causes of lymphadenopathy such as various viral infections or cat-scratch disease can often be differentiated by the discrete and often tender adenopathy they produce and more usually involvement of other chains of nodes. Herpes genitalis may present with lymphadenopathy. Bacterial lymphadenitis may mimic LGV, but sites of invasion should be recognized, and, of course, in these instances, bacteria will be recognized in node aspirates. Lymphoma and leukemia are also a part of the differential diagnosis.

The laboratory diagnosis is most definitively made by NAAT or by tissue culture of bubo aspirate. However, if the node is necrotic enough to rupture spontaneously, it may be too late to recover active organisms. (In the past, yolk-sac culture or mouse inoculation was used for diagnosis, but tissue-culture methods are more sensitive.)

The Frei antigen—a skin test made of a partially purified elementary body suspension of yolk-sac-grown LGV—was used widely for diagnosis but it is not available in the United States today. However, cell culture isolation and serological methods have supplanted the Frei test. The poor sensitivity and specificity and the delay in development of the latter test have made it obsolete.

As with other chlamydial infections, the definitive serological method is the MIF method. However, in the case of LGV, as with psittacosis, CF antibodies usually result from infection within a few weeks of the initial infection and characteristically result in high titers ($>$1:64). The CF test is group specific and may be performed with either LGV or psittacosis yolk-sac- or cell-culture-prepared antigens. Although cross-reactions with the oculogenital trachoma strains would be expected, the general insensitivity of the CF test results in few patients with the oculogenital syndromes who have detectable CF antibody, and then usually at low titer.

8.4. Psittacosis

8.4.1. Clinical Features. The most common human manifestation of infection is an atypical pneumonia. It may be a relatively mild systemic disease (like mycoplasmal pneumonia) or a more severe one (like influenza). An alternative presentation is an acute septic-like illness with fever and chills and little pneumonitis. Liver and spleen enlargement may occur (particularly in the latter toxic form). Without treatment, fever may extend into the second week, and before chemotherapy, the case-fatality rate averaged 20%, being higher in those 50 years of age and older. With

apparent recovery, relapse may occur later —sometimes a matter of years following the original infection. In the 1,136 cases reported in the United States in 1975–1984, pneumonia occurred in 54%.[64] Other common symptoms are headache, weakness, and myalgia. Rarely, manifestations such as arthralgia, meningiomas, abdominal pain, photophobia, pleuritis, or pericarditis may result.

8.4.2. Diagnosis. Serological diagnosis is most often employed. The CF test (insensitive for trachoma or oculogenital disease) is sensitive enough for this disease. The usual criterion is a fourfold increase in titer from acute to convalescent sera, but single titers above 1:64 are highly suggestive. *Chlamydia psittaci* can be isolated in yolk sac in embryonated eggs, in mice, or in tissue-culture systems. The best source for isolation is sputum or bronchial aspirate. Whole blood (heparinized or citrated), serum, or plasma may occasionally yield the agent. Isolation is more common in the second week of disease.

8.5. *Chlamydia pneumoniae* Infections

8.5.1. Clinical Features. The limited observations to date suggest that *C. pneumoniae* organisms cause a range of respiratory disease from pharyngitis and otitis media through bronchitis to pneumonia.[20,65] Illness is more frequent in school-aged children and young adults. It is also apparent that the majority of infections are of low-grade manifestation or are asymptomatic. There appears to be some tendency for more severe manifestations to be seen in the adolescent or young adult. In general, the disease is mild. Onset of illness is gradual and symptoms are often extended over a number of weeks. In all these characteristics, *C. pneumoniae* disease is very similar to *M. pneumoniae* disease.

Pharyngitis (often with laryngitis) appears to precede lower respiratory disease in most cases, often by a week or more, and often gives the impression of a biphasic illness. By the first clinic visit an average of 13 days has elapsed from the onset of symptoms and the patient is often afebrile. Sinusitis is more often present than in *M. pneumoniae* infections. Chest findings are common on auscultation at this stage. With lower respiratory tract symptoms, the chest radiograph almost always demonstrates pneumonia. There is commonly a single subsegmental lesion, although more extensive involvement is sometimes found. Of pneumonia patients, 84% were outpatients.[65] In hospitalized patients, three retrospective serologic studies have found 6–10% of community acquired pneumonia to be due to *C. pneumoniae* infection.[20] In this study there were no clinical or radiologic features that differentiate this etiology. In this setting the mean age was in the sixth decade and there was often preexisting chronic illness. This reflects the finding that the disease is commonest in youth, but more severe in the elderly. Bronchospasm and the onset of asthma have recently been associated with *C. pneumoniae* infection, but these findings need to be corroborated.[88]

Many research studies have shown an association between *C. pneumoniae* infection and atherosclerosis. The original observations were seroepidemiologic studies that found high titers of antibody to *C. pneumoniae* in atherosclerotic patients. The seroepidemiologic data associating *C. pneumoniae* infection with coronary artery disease led to a very broad-based research approach attempting to associate *C. pneumoniae* infection with atherosclerosis. Many studies found that the organism was present in coronary artery plaque. This was demonstrated by a number of different procedures including PCR, in vitro hybridization, cytology, electron microscopy, and isolation of the organism from atherosclerotic tissue. Animal models showed that *C. pneumoniae* infection could induce lesions in arteries or could speed the development of these lesions in animals selected for the ability to develop such lesions due to dietary or hereditary considerations.

A number of relatively small trials of antibiotic treatment suggested that antichlamydial therapy had a beneficial effect in preventing the development of secondary coronary events. However, more definitive studies using either azithromycin or gatifloxacin that were well powered to detect the efficacy of antibiotic treatment for prevention of secondary coronary events failed to confirm results of the smaller studies. The risk reduction was essentially negligible in these large studies that involved many thousands of patients. Thus, at this point one would consider that routine antibiotic treatment for *C. pneumoniae* for the prevention of secondary coronary events is not indicated.[89]

However, it must be stated that the failure of antibiotic treatment to prevent secondary coronary events does not prove or disprove the hypothesis that *C. pneumoniae* causes coronary artery disease. It is likely the only way to prove that hypothesis would be if an effective vaccine were to be developed and an appropriate long-term trial could be done to actually prevent *C. pneumoniae* infection and allow such infection to develop in a placebo-vaccinated group. There are several theoretical reasons why the antibiotic treatment following the coronary event would not be beneficial even if *C. pneumoniae* were the cause of the disease. First, it is possible that the role of C. *pneumoniae* is in early-stage disease and not late-stage disease. Second, it is not clear that these drugs which do have an antibacterial effect on the organisms in vitro, perhaps even in epithelial cells, would have the same efficacy in treating infection *in situ* (presumably, in histiocytes or macrophages).

The response to antibiotics is not striking. In vitro studies show maximum sensitivity to tetracyclines and erythromycin. Maximum levels of antibiotics are needed for prolonged periods, usually for at least 14 days. New longer acting macrolides such as azithromycin show promise, but there have been no controlled trials reported.

There are a number of other diseases that researchers have claimed, or speculated, are caused by *C. pneumoniae.* But because of the very high infection rate and the high prevalence of antibodies it is very easy to demonstrate *C. pneumoniae* infection in essentially any disease one would like to study. It does not mean that there is either a specific association or a causative relationship between that infection and the disease. Thus, it has been difficult to understand many of these claims. This combined with the problems in diagnostic tests and reproducibility from laboratory to laboratory, and one winds up dividing the literature into those who are believers and those who are non-believers.[90]

One area where this is clearly true is the area of neurologic disease due to *C. pneumoniae*. A number of studies have shown that the organism is present in the central nervous system. Whether this actually reflects true infection of the brain, for example, or whether it is an incidental finding or an epiphenomenon associated with chlamydial infection of blood vessels in the brain is certainly not clear. There are studies suggesting that *C. pneumoniae* could cause multiple sclerosis and Alzheimer's disease. While positive results have been reported from more than one laboratory for both of these conditions it is far from clear that there is any etiologic relationship.

8.5.2. Diagnosis. Isolation of *C. pneumoniae* has been difficult because the organism failed to adapt readily to commonly used cell cultures for other Chlamydiae. The use of HL cells has improved this situation.[32] The organism can be isolated from respiratory specimens, but the yield from pharyngeal specimens (often all that is available) is not great. Lower respiratory tract specimens yield better results. A major problem in understanding chlamydial diseases caused by *C. pneumoniae* is the lack of sensitive, reproducible diagnostic tests. There are many home-brew NAAT procedures that have been used and none of them have taken the lead as the test of choice. This lack of standardization has a corollary and that is lack of reproducibility. Some laboratories have found certain procedures easy to do and report having successful results with demonstration of the organisms quite easily. Others try the same procedure with negative results. The ultimate question is, are those who claim success actually getting true positives; or are they dealing with false-positive results or laboratory contamination; or are those who report negative results actually failing to perform these assays in the correct way and thus getting poorer results? This has been a particular problem with PCR tests, where results are highly divergent.[91,92] The serologic test of choice is the micro-immunofluorescence (MIF) test and it is highly dependent upon the expertise of the reader and is not a procedure that is easily mastered. It has become the standard test for serology. There are commercially available serologic kits whose performance is uncertain. Even with MIF, this test in the best of hands fails in the most crucial aspect of the diagnosis of chronic infection. For acute infections, fourfold seroconversions and high IgM titers are markers of recent infection. The choice of a single high titer as an indication of acute infection is in fact a compromise for epidemiologic purposes, and it is not proof of acute infection at all. IgA antibodies, because they are short-lived in other settings, have been proposed by some as markers of chronic infection, but other studies have found that IgA antibodies are largely a trailing indicator of IgG and thus are not markers for either acute or chronic infection. The bottom line is that there is no simple way of diagnosing a chronic infection.

The micro-immunofluorescence test (MIF), developed for *C. trachomatis* is the only sensitive and specific test available, and is useful for serologic studies.[14] The test is demanding and is available in only a few research and commercial laboratories. Two patterns of response have been described. The first is one of slow development of IgG and IgM antibodies over 1–2 months, with subsequent loss of IgM antibody. This would appear to be a primary type of response. The second pattern is one of rapid IgG response in the absence of any IgM response and is more often noted in older individuals. This is more characteristic of a secondary or reinfection response.

Complement-fixing (CF) antibodies are not specific among *Chlamydia* species. The test is often used for the diagnosis of psittacosis. In the absence of good epidemiologic evidence for *C. psittaci* infection, a positive CF test is more likely to be due to *C. pneumoniae*. CF antibody response is found in two-thirds of patients in whom a primary response MIF pattern can be measured. No CF antibody is measured in reinfection pattern sera. Thus CF antibodies may be useful in screening for primary infection (particularly in younger patients). They are not useful in the diagnosis of reinfections or for seroepidemiologic studies.

9. Control and Prevention

9.1. Trachoma

9.1.1. General Concepts. Elimination of trachoma has occurred in large segments of the world, not as a result of specific control programs, but following the changes in

lifestyle that accompany a raised standard of living. However, in those areas of the world where trachoma remains hyperendemic, such changes are not likely to occur soon. Therefore, much national and international effort has been expended (with significant contributions from the WHO) on trachoma control programs. In the last few decades, trachoma control programs have been based almost entirely on the mass application of locally applied antibiotics. The results have been far from encouraging. After initial response, trachoma returns to become endemic again.

Recognition that community-wide treatment with azithromycin could dramatically reduce the prevalence of chlamydial infections has revolutionized our approach to trachoma control.[93] A single dose of azithromycin has become the treatment of choice for genital chlamydial infections and was shown to be effective in treating trachoma cases. Community-wide treatment has been incorporated into a control program that is aimed at the ultimate elimination of blinding trachoma as a public health program by the year 2020. The International Trachoma Initiative (http://www.trachoma.org) has been organizing this program with massive support from the manufacturer of azithromycin who has been donating all of the antibiotics and private charitable organizations, non-governmental organizations (NGOs), and the WHO. The program is based on what is being called the "SAFE" strategy:

- S—Surgical repair of lids already damaged by trachoma to prevent the future development of blindness
- A—Community-wide treatment with Azithromycin in trachoma endemic areas.
- F—Facial cleanliness to minimize the transmission of *Chlamydia* after the prevalence of infection has been reduced by antibiotic treatment.
- E—Environmental improvements, also aimed at minimizing the transmission of *Chlamydia* through the provision of latrines (aimed at controlling flies) and safe water supplies.

9.1.2. Antibiotic and Chemotherapeutic Approaches. Community-based antibiotic treatment programs with oral azithromycin treat both ocular and extraocular chlamydial infection, and those with clinically active trachoma, as well as asymptomatic carriers.

9.1.3. Immunization. Soon after *C. trachomatis* was first recovered in eggs, laboratories in London, Boston, and Taipei (later Seattle) began developing vaccines for trachoma.[47] Even though reinfection can readily occur in humans following prior trachoma, it was found in monkey models that hyperimmunization with vaccine could prevent reinfection, at least under the somewhat artificial environment of an animal trial. In one instance, a similar result was obtained in blind volunteers. However, each of the candidate vaccines was made with relatively crude whole-organism preparations, and all of them gave only fleeting protection. Even more discouraging was the finding that both in animal experiments and in human trials, subsequent rechallenge might result in more severe disease than occurred in rechallenge after natural infection (a phenomenon that has also been seen in other experimental vaccines of low antigenic potency). At that point, all vaccine trials were abandoned, pending further study of the molecular biology of the organism, with the hope of discovering antigenic subunits that might result in a more permanent immunity. Such efforts are about to move from the laboratory bench to animal testing.

9.2. Oculogenital Diseases

9.2.1. General Concepts. Since these are STDs, control measures are similar to those for gonorrhea and syphilis. *Chlamydia trachomatis* should be sought by culture or antigen detection methods in persons with STD complaints or history. Current recommendations include treatment of all persons with urethritis (gonococcal or nongonococcal), mucopurulent cervicitis, pelvic inflammatory disease, and epididymitis in men under age 35 years as if they had both gonococcal and chlamydial infection.[58] They should be treated with a penicillin-like antibiotic (or alternative where penicillin-resistant strains exceed 3%) and a tetracycline antibiotic (or erythromycin in pregnant women). Other individuals recommended for *C. trachomatis* treatment are asymptomatic contacts to patients with the four syndromes mentioned above and others with established gonococcal or chlamydial infections. Similarly, sexual partners of persons with gonococcal infection should be treated. All of these could be treated without a positive laboratory test if diagnostic laboratory sources are limited. Then diagnostic screening can be saved for highest risk groups. Current recommendations are that all sexually active women between ages 16 and 25 years should have annual tests for chlamydial infection.[58] Those found to be infected should be retested more frequently (3–4 months interval). When possible, contact tracing should be pursued. Both cases and contacts should be treated concurrently and should avoid intercourse (or use condoms) during treatment. In pregnant women, culture and treatment are indicated to protect against postpartum maternal and neonatal consequences.

9.2.2. Prophylaxis. In infected males and nonpregnant females, treatment with azithromycin or doxycycline eliminates infection and therefore provides prophylaxis against the complications of that infection.[58]

Treatment of women during pregnancy or immediately postdelivery will eliminate infection and may protect against postpartum fever and endometritis. Furthermore, for the

treatment to be effective during pregnancy, the sexual partner must be treated simultaneously to prevent reinfection. Azithromycin is considered the drug of choice (1 g oral). If further study elucidates prenatal fetal or maternal complications of infection, or both, routine first-trimester cervical culture and treatment of positive women and partners may be a theoretically useful prophylactic procedure.

For infants, effective prevention of chlamydial disease occurs by treating mothers and partners prior to delivery.[94] Silver nitrate prophylaxis is ineffective against Chlamydiae; topical erythromycin or tetracycline eye prophylaxis at birth for the prevention of inclusion conjunctivitis may reduce it somewhat, but are not highly effective and will not prevent pneumonia.

Antibiotic resistance of *C. trachomatis* has not been shown and is not currently a clinical problem.

9.2.3. Immunization. No immunization for oculogenital diseases is available.

9.3. Lymphogranuloma Venereum

9.3.1. General Concepts. Prevention of LGV depends on the recognition and treatment of infected persons. Problems exist in both these aims. Although classic cases of LGV are easily recognized, relapse of old disease may occur, particularly in debilitated persons. Furthermore, the occurrence of relatively silent disease in anogenital carriers, particularly in sexually active homosexuals, and the difficulty in availability of simple diagnostic tests result in rather persistent endemic foci. For LGV, unique among other chlamydial diseases, a specific highly effective method of chemotherapy is also lacking. Sulfonamides were classically used, but more commonly today, tetracycline therapy is preferred. The major difficulty is in assessing the effect of therapy, particularly in treatment of the embryonic or tertiary stage.

9.3.2. Prophylaxis and Immunization. No prophylaxis or immunization for LGV is available.

9.4. Psittacosis

9.4.1. General Concepts. Control of psittacosis depends on control of avian sources of infection. Control of the 1929 pandemic of psittacosis was accomplished by bans on the importation of psittacine birds. This was followed by a method of certification of clean flocks, but many of these efforts were damaged by poor supervision, smuggling, and contamination of susceptible birds from clean flocks during shipment. An effective method for controlling psittacosis in parakeets has been developed—a chlortetracycline-impregnated seed. Current requirements for quarantine of imported birds are for 30 days of treatment. This is probably not enough, and it has been proposed that this be extended to 45 days. It is probable that some birds have suppressed infections that reactivate during shipment following quarantine. The remaining problems are ones of assuring more adequate response to control regulations.

The control of occupational disease in the poultry-processing industry appears to have been successful in recent years, although there is no developed public health program. The epidemics in 1974 served to alert the industry to the problem, and the infection in poultry flocks tends to be rapidly recognized and treated. It is also probable that human disease continues to occur, but is quickly recognized, treated, and not reported in these workers.

9.4.2. Prophylaxis and Immunization. There is no prophylaxis in humans, and although experimental psittacosis vaccines have been developed in birds, they are not cheap enough to replace antibiotic treatment of birds. There is no need to develop human vaccine for such an infrequent disease.

10. Unresolved Problems

10.1. Trachoma

For trachoma, there is no problem of diagnosis, since clinical recognition is relatively simple. Effective treatment is available. A vaccine would be useful, but development of one depends on a greatly expanded understanding of the immunology and molecular biology of this agent.

10.2. Oculogenital Diseases

10.2.1. Epidemiology and Disease Spectrum. There have been no significant additions to our knowledge of the disease spectrum in recent years. Syndromes such as otitis media, prostatitis, diarrhea, or pharyngitis have not been shown to be caused by *C. trachomatis*. Reports that claimed to find a significant contribution of *Chlamydia* to pharyngitis were based on serological evidence. It is now clear that this phenomenon and the unexplained increment of antibody in early childhood were both a function of cross-reacting antibody to *C. pneumoniae* organisms.

10.2.2. Diagnostic Tests. Chlamydial cultures and serologic techniques are time consuming and expensive; they are not very useful as screening techniques. Amplified DNA technology is extremely sensitive and specific, even on urine specimens and vaginal swabs.[30] As these tests become more available and less expensive they bring the potential of mass screening with non-invasively collected specimens

(first catch urines, self-collected vaginal swabs) will revolutionize control approaches. With the use of these techniques in high-risk young women and men and with single-dose azithromycin therapy now available there is the prospect for detection and elimination of asymptomatic infection, and thus an opportunity for major reservoir reduction, and disease control.

10.2.3. Host Response. Data from individuals with asymptomatic infection have shown that infection may persist in the presence of both serum and local antibody. The contribution of other arms of the immune response to clearance of infection is not clear, but certainly cell-mediated immunity and gamma interferon appear to have important roles in clearing infection.

10.2.4. Control. Control of oculogenital infections due to *C. trachomatis* constitutes a large-scale public health problem. As has been discussed in other sections, true control of these infections will require definition of all the illnesses caused by *C. trachomatis*, and all modes of transmission of infection, coupled with the development of rapid and easily applicable diagnostic techniques.

10.3. Lymphogranuloma Venereum

The outstanding problem in this case, as with the oculogenital syndromes, is the development of a rapid, inexpensive diagnostic method. In this instance, further study of optimal chemotherapy is also needed.

10.4. Psittacosis

In this instance, it would seem that the basic tools are at hand. A vaccine for birds or a more effective antibiotic feed method that would assure elimination of avian infection in less than 30 days would assure better cooperation by the industry.

10.5. *Chlamydia pneumoniae* Infections

There are many research needs for this agent. Culture and serological methods need to be further adapted for this organism. Basic epidemiological and natural history studies are needed. Although serological studies indicate a ubiquitous agent, there appear to be periods of occurrence and absence in given locations. The transmission of the organism, its infectivity, and incubation period need to be defined. Methods of treatment, control, and prevention require extensive research.

It is clear that *C. pneumoniae* can induce a chronic/latent infection. It is not clear whether these persistent infections involve true latency (i.e., persistence of a non-replicating form) or whether this is simply a slower form of replication. It is clear that chlamydial species do a number of things to cells that help them to persist. The chlamydial inclusion avoids fusion with lysosomes, and the infection inhibits host-cell apoptosis, thus allowing the organism when it is in a superficially shorter-lived cell to persist for a longer period of time.

Another important issue is, how do we treat these *C. pneumoniae* infections—particularly when they are chronic infections? If the organism is persisting in a non-replicating stage then those antibiotics which are active against metabolically active bacteria would have no effect. It is not clear that such a chronic persistent infection actually exists; having said that, if this infection does exist, the therapeutic implications are daunting.

Of course, the most important question here is still, does this organism cause coronary artery disease? The failure of treatment trials does not answer that basic question.[89]

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CHAPTER 12

Cholera

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1. Introduction

Cholera is a severe diarrheal illness caused by certain types of *Vibrio cholerae*, that can lead rapidly to dehydration and death. Although other organisms occasionally cause similar illness, the term "cholera" is reserved for illness caused by infection with toxigenic strains of *V. cholerae* O1 or O139, whether the symptoms are mild or severe. Epidemic cholera has appeared relatively recently on the global stage, spreading throughout the world early in the 19th century and causing severe epidemics in the crowded cities of the newly industrializing Europe. Epidemic cholera has since recurred in massive multicontinental pandemics. These pandemics had a profound impact on the development of public health, stimulating the establishment of standing health departments, ongoing infectious disease surveillance, and swift and effective public health response to epidemics. The discovery that cholera was associated with contaminated municipal drinking water spurred the "sanitary revolution," leading to modern water and sewage treatment systems and the control of many diseases in the developed world. Now as then, epidemic cholera continues to goad countries in the early phases of industrialization to develop adequate water and sanitation infrastructures.

Treatment for cholera is simple and highly effective. Rapid replacement of the lost fluids and electrolytes with oral and intravenous solutions can reduce mortality from 25–50% to less than 1%. The organism can be transmitted through contaminated drinking water and through a variety of foods. The specific vehicle of transmission can vary from place to place and during the course of a single epidemic, complicating epidemic control efforts. Efforts to improve drinking water safety and food sanitation can be accelerated by the appearance of epidemic cholera, and prevention can ultimately be achieved by improving the sanitary infrastructure in susceptible areas. Some simple prevention strategies may prove sustainable and effective. Natural infection gives immunity that wanes over time, in the absence of repeated exposure, as do existing and experimental vaccines.

In the 1990s, two events dramatically changed the epidemiology of cholera. The appearance of epidemic cholera in Latin America in 1991, after an absence of over 100 years, stimulated improvements in public health and sanitation throughout the hemisphere. In 1992, a new strain of *V. cholerae*, called O139 Bengal, appeared in India and spread to several countries in south Asia (Figure 1), causing a major epidemic in an adult population that was already largely immune to cholera caused by *V. cholerae* O1 strains. Although the O139 strain has since been relatively quiescent, it may represent the beginning of a new pandemic. The sudden appearance of this serogroup is a case study in the evolution of bacterial serotypes and in the emergence of new pathogens in general.

2. Historical Background

The history of cholera is linked to the early modern era of industrial civilization. Epidemic cholera is not described in early Mediterranean or Middle Eastern medicine texts, though accounts compatible with cholera exist from medieval India.[1] The disease has a homeland in the

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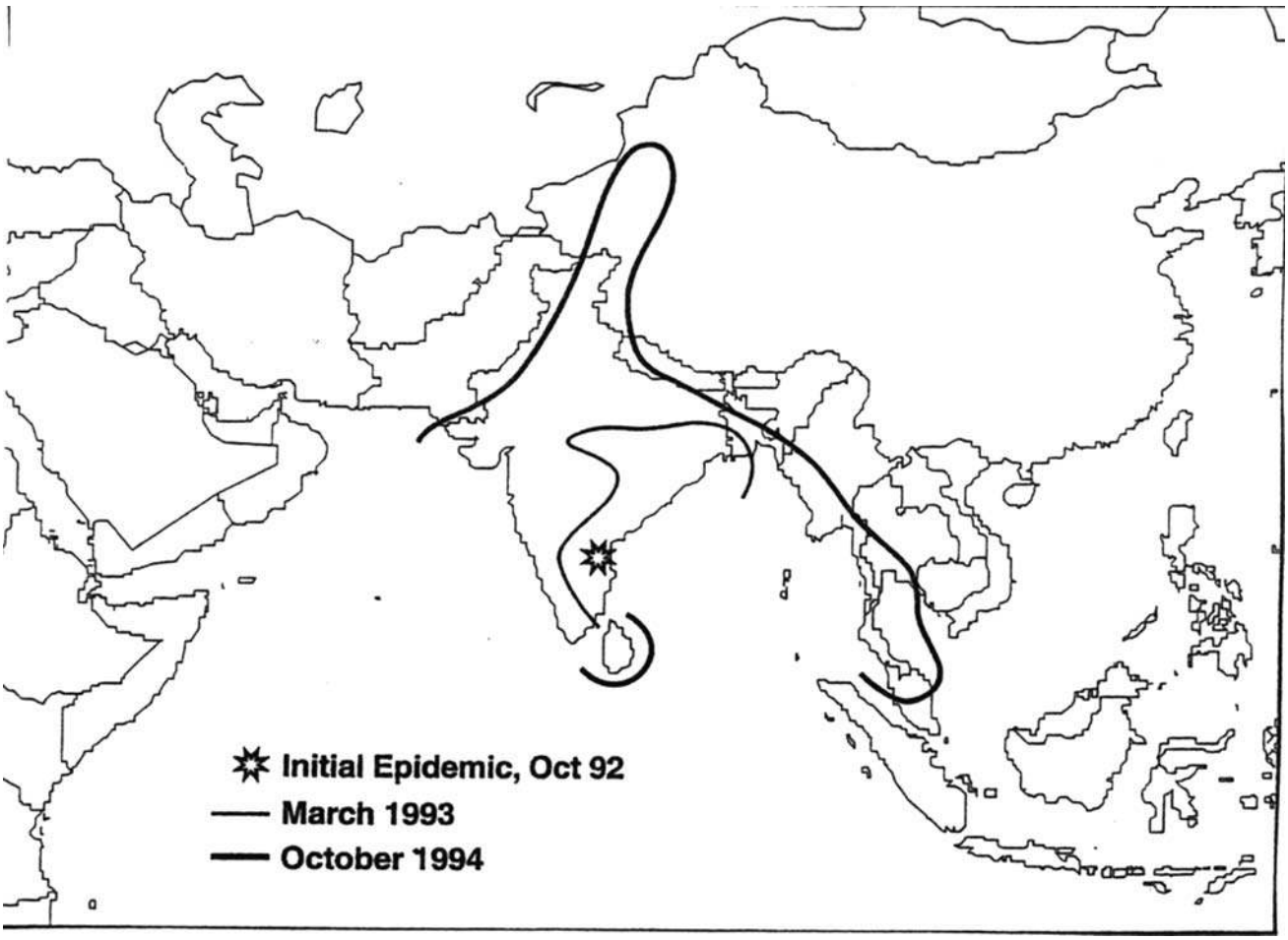


Figure 1. Spread of the epidemic of *V. cholerae* O139 infections in Asia.

Bengal basin, the delta of the Ganges and Brahmaputra Rivers. The vibrio is well-adapted to the estuarine environment and may have been established for many centuries in this natural reservoir, occasionally infecting those who drank or consumed raw shellfish from those waters, in an ecological pattern resembling that of many vibrios, including *V. cholerae* other than O1 or O139.

Beginning in 1817, cholera spread from Bengal to other parts of the world in repeated pandemic waves that affected virtually all of the inhabited world. Precisely when each of these waves began and ended is a matter of historical judgment. The most frequently cited account is that of Pollitzer,[1] who divided them into six major pandemics between 1817 and 1923 (Table 1). The global spread of cholera was hastened by the establishment of European colonies in Asia, which led to increased international trade, and by the steamship, which shortened intercontinental travel times. The Hajj, or annual Muslim pilgrimage to Mecca, helped to disseminate cholera in pandemics four–six.[1] Cholera was likely to have been a regular feature of life in Asia in the 19th century. However, the impact was particularly severe in rapidly growing European cities, where huge lethal epidemics occurred. In those cities, immigrants from the countryside crowded into new slums, seeking employment in the new mills and factories of the industrial revolution. Those Dickensian slums bear a striking sociological resemblance to the barrios, favellas, and periurban slums that ring cities in the developing world today. Investigations of the 1854 epidemic in London by John Snow[2] showed that the cholera death rate in homes served by the Southwark and Vauxhall Water Company was 31 per 1,000 houses, 8.5 times higher than the death rate in homes served by the competing company, which was 3.7 per 1,000 houses. Although neither company provided treated water, the former drew water from

Table 1. Cholera Pandemics Since 1817[a]

No.	Years	Origin	Pandemic organism
1	1817–1823	India	?
2	1829–1851	India	?
3	1852–1859	India	?
4	1863–1879	India	?
5	1881–1896	India	*V. cholerae* O1, classical
6	1899–1923	India	*V. cholerae* O1, classical
7	1961–present	Sulawesi, Indonesia	*V. cholerae* O1, El Tor

[a]Adapted from Pollitzer.(1)

the Thames below the city sewage outlet, while the latter was supplied from the river above the city. An outbreak of cholera was linked to consuming water drawn from one particular and celebrated pump, located in Broad Street. These investigations are classic examples of the application of the epidemiological method even in the absence of a defined microbiological cause. In 1890, similar observations were made in Hamburg, where the incidence of cholera was 34 per 1,000 compared with 3.9 per 1,000 in surrounding areas that were not supplied by water from the River Elbe.(1) These observations catalyzed efforts to provide safe drinking water and adequate sewer systems in Europe and North America.

In 1884, the microbiological cause of cholera was described by Robert Koch(3), who investigated cholera in Egypt during the fifth pandemic. The strains he described are what we now refer to as the "classical" biotype of *V. cholerae* O1, the cause of pandemics five and six.

Epidemic cholera reached the United States on several occasions.(4) In 1832, the nation responded with prayer and panic, and many believed that the disease was a divine retribution afflicting the wicked. Some cities concealed cholera cases, while others ineffectively banned the transit of goods or passengers from suspected cholera-affected areas. By 1866, a more rational approach prevailed. Temporary boards of health were created in many cities, and began to conduct surveillance, to hospitalize cholera patients, and to take measures to improve the municipal water supply. After 1880, in the fifth and sixth pandemics, resurgent European epidemics again threatened the United States. The value of surveillance was widely appreciated, and many local boards of health became permanent institutions. Laboratory diagnosis was used to identify cases. Many municipal aqueducts and protected watersheds date from that era. Large-scale epidemic spread did not occur, suggesting that even then the public health infrastructure was sufficiently advanced to protect the population.(1)

After the sixth pandemic, cholera entered a curious 50-year lull, and again was largely restricted to the Bengal homeland. This lull broke in 1961, when epidemic cholera appeared in Sulawesi, Indonesia, and then disseminated rapidly.(5) This seventh pandemic was caused by the El Tor biotype of *V. cholerae* O1. It spread rapidly across Asia and into Africa by 1970 (Figure 2).

Events in the 1990s changed the epidemiology of cholera. In 1991, after a greater than 100-year absence

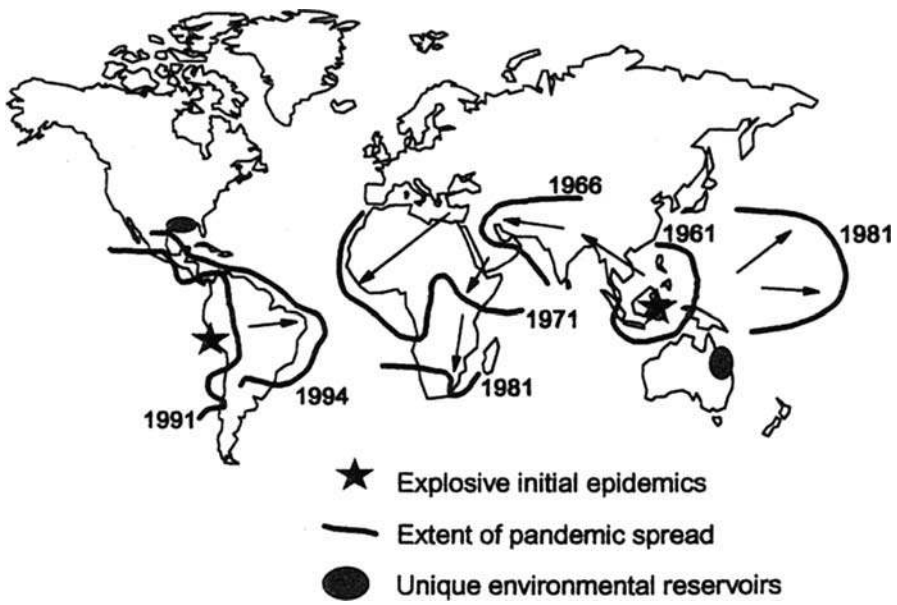


Figure 2. Global spread of the seventh cholera pandemic, 1961–1994, and recently defined areas with environmental reservoirs of toxigenic El Tor *V. cholerae* O1.

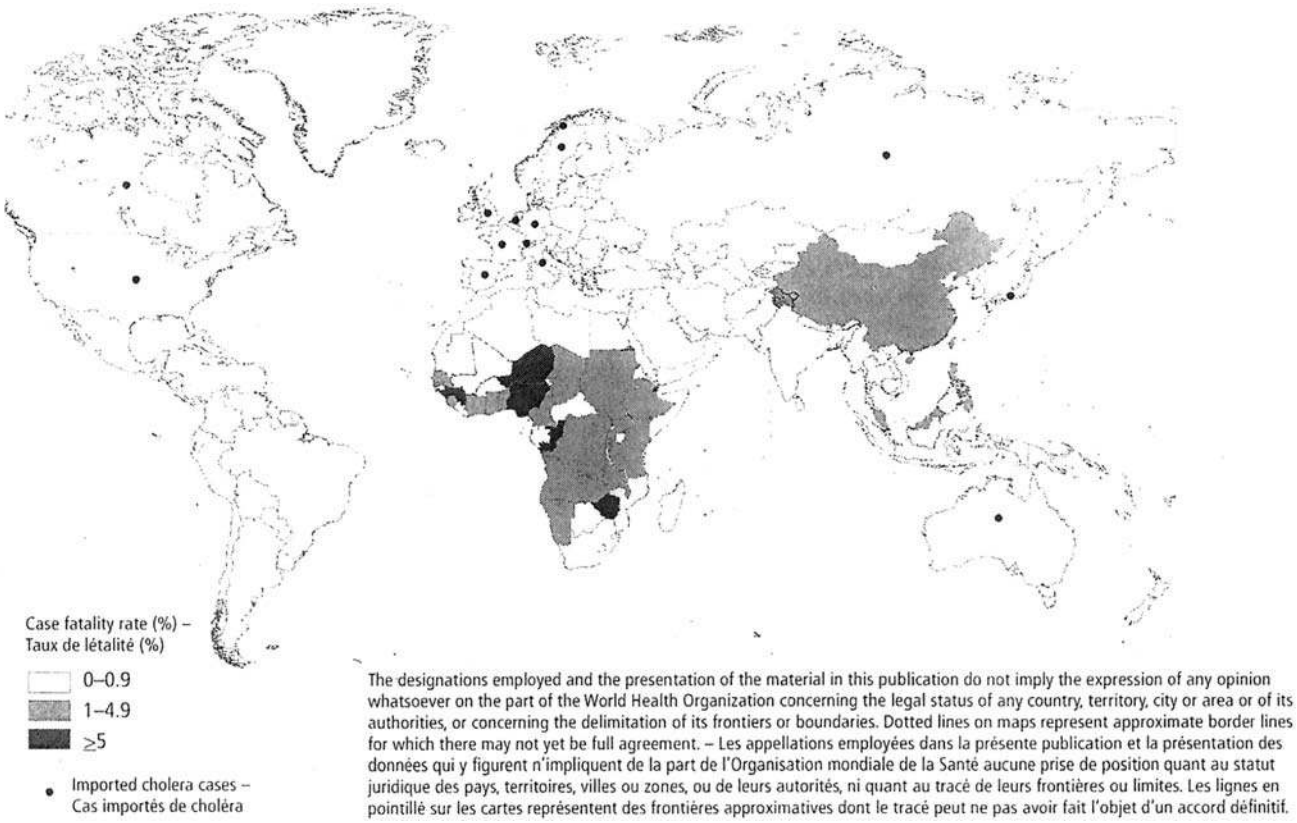

Case fatality rate (%) –
Taux de létalité (%)
0–0.9
1–4.9
≥5
Imported cholera cases –
Cas importés de choléra
The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. – Les appellations employées dans la présente publication et la présentation des données qui y figurent n'impliquent de la part de l'Organisation mondiale de la Santé aucune prise de position quant au statut juridique des pays, territoires, villes ou zones, ou de leurs autorités, ni quant au tracé de leurs frontières ou limites. Les lignes en pointillé sur les cartes représentent des frontières approximatives dont le tracé peut ne pas avoir fait l'objet d'un accord définitif.

Figure 3. Countries/areas reporting cholera cases in 2007, World Health Organization.[8]

of epidemic cholera, a variant E1 Tor strain appeared in Peru in a new epidemic wave that spread swiftly through Latin America. By November 1991, cholera was reported in 12 Latin American countries, affecting over 330,000 individuals.[6] Additional cases were subsequently reported from other Latin American countries during this epidemic and accounted for over 65% of all reported cases globally in 1991.[7] More recently, perhaps due to improvements in public health and sanitation, the reported cases of cholera have dramatically decreased in that region.[7]In 1992, a new serogroup of *V. cholerae*, called O139 Bengal, appeared in India and caused a major epidemic in a population that was already largely immune to cholera caused by *V. cholerae* O1 strains. *Vibrio cholerae* O139 possesses epidemic potential but after a brief excursion into South East Asia, it has been largely restricted to south Asia for unknown reasons. In the mid-1990s, following the dissolution of the Soviet Union, epidemic cholera appeared in the decaying cities of central Asia, revealing the importance of a sound public health infrastructure. Epidemic cholera continues to exert a major influence on the global development of society and public health, disproportionately targeting impoverished areas lacking in improved public health and sanitation systems. The World Health Organization (WHO) continues to receive reports of cholera from most regions of the world; data from central Asia, central America, and Latin America were not available in 2007 (Figure 3). In 2007, the WHO confirmed more than 177,963 cases (Figure 4) and 4031 deaths from cholera. These numbers include 45 confirmed cholera epidemics, 83% of which occurred in Africa.[8]

3. Methodology

3.1. Sources of Mortality Data

Cholera was the first disease for which modern public health surveillance was organized. Thus, cholera bears the code 001 in the international classification of diseases and, along with plague and yellow fever, cholera is an internationally notifiable disease. The cholera epidemics of

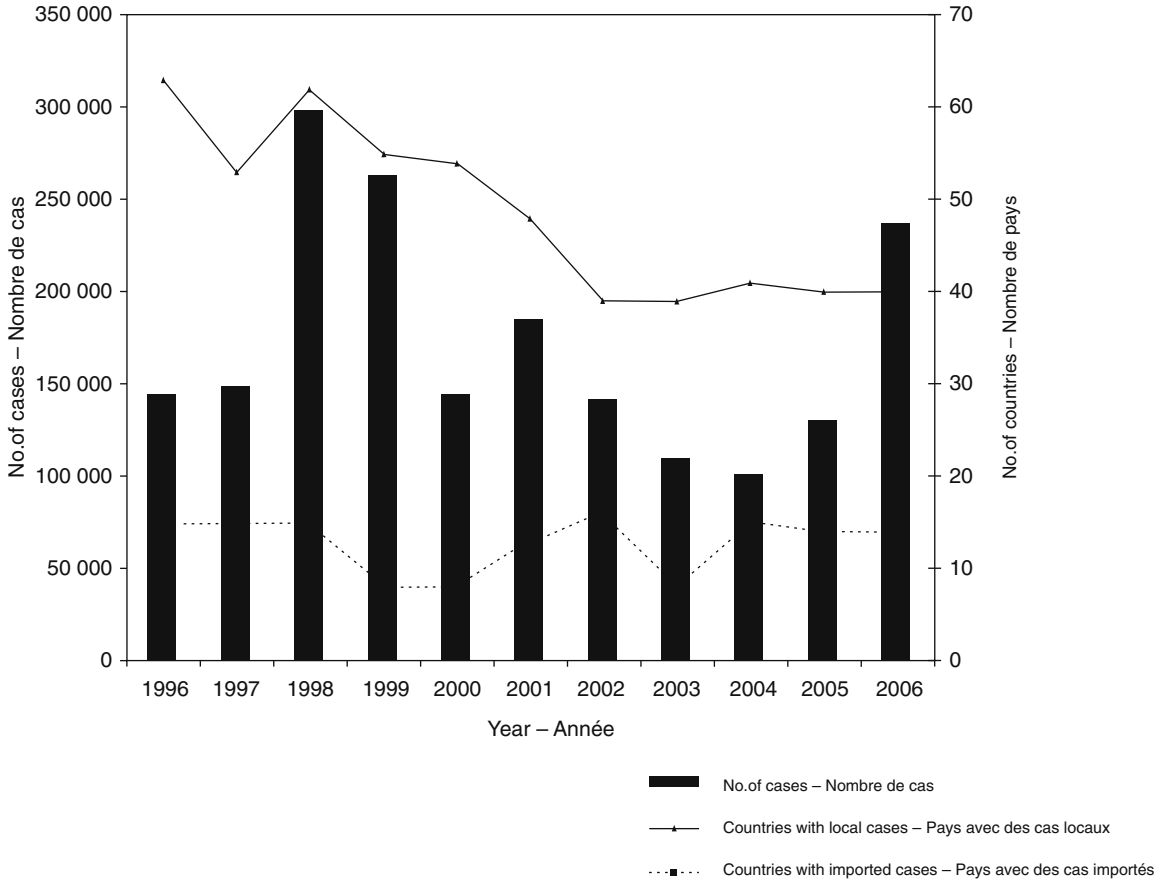


Figure 4. Countries/areas reporting cholera and cases reported to World Health Organization by year, 1997–2007.[8]

the 19th century prompted the development of the current International Health Regulations (IHR). The IHR have recently been revised to include the notification of diseases which constitute a "public health emergency of international concern". [9] Cholera continues to meet this definition. These regulations, which were adopted in 2005, were implemented beginning in 2007. Many countries conduct routine surveillance for cholera and report the results to the World Health Organization (WHO). Political concerns, fear of inappropriate trade embargoes or loss of tourism, and other imagined penalties cause some countries to conceal the presence of cholera. In particular, Egypt, Thailand, and Bangladesh have for years officially denied the presence of cholera, referring instead to "severe diarrheal illness." In many other countries, particularly in Africa, vital statistics are not routinely gathered, disease reporting is limited, and many cases and deaths go unreported. Thus, official global statistics on cholera provide a very incomplete picture.

The definition of a cholera death varies greatly. The definition recommended by the Pan-American Health Organization is "death within 1 week of onset of diarrhea in a person with confirmed or clinically defined cholera."[10]

3.2. Sources of Morbidity Data

Although cholera is a notifiable disease in most countries, the actual number of cases reported is far less than the number that occur. In parts of South Asia, infection with *V. cholerae* is probably nearly universal, yet no cases are reported. The definition of a case used for surveillance purposes varies considerably.[10] The WHO definition categorizes cases as suspect or confirmed and is dependent on whether cholera is endemic to the region. A suspect case of cholera in a non-endemic region occurs when "a patient, 5 years of age or older, develops severe dehydration or dies

from acute watery diarrhea". [11] In an endemic region, a patient older than 5 who "develops acute watery diarrhea, with or without vomiting" meets the suspect case definition. A confirmed case is dependent on isolation of either *V. cholerae* O1 or O139. Regardless of laboratory confirmation, all cases of cholera are included in WHO surveillance data. The precision of the surveillance definition is improved by excluding those persons under the age of 5 years, given the numerous other causes of pediatric diarrhea, at the expense of counting younger patients. [12] In Peru, the positive predictive value of this simple case definition was better than 80% early in the epidemic.[13]

When cholera is rare, other severe diarrheal illnesses will be more common than cholera, which renders the clinical definition too nonspecific for public health use. Therefore, in most industrialized nations, the surveillance case definition of cholera depends on laboratory isolation of toxigenic *V. cholerae* O1 or O139, or demonstration of diagnostic antibodies.[14] Because many other *V. cholerae* cause occasional illnesses, without the same public health implications, it is important in the setting of a sporadic infection to verify the serogroup of *V. cholerae* as O1 or O139 and the toxigenicity of the organism before calling the illness cholera.

Where cholera is common, case reporting and public health response should not be limited to laboratory-confirmed cases. It is not necessary to expend scarce laboratory resources confirming many thousands of cases during an epidemic; the most useful roles of the laboratory are to initially identify the organism causing the epidemic, to monitor changes in antimicrobial resistance during the epidemic, and to establish that an epidemic is over.[15]Additionally, laboratories play an integral role in identifying the source of infection and extent of the epidemic. As such, microbiologists, in addition to epidemiologists, should be included in epidemic response and control teams.

3.3. Surveys

For many years, the International Center for Diarrheal Disease Research in Bangladesh has maintained surveillance of urban and rural populations, defining the epidemiology of cholera in that area. Population-based surveillance has also been conducted in Kisumu, Kenya. [16]

Serological surveys conducted in the epidemic setting show that the actual number of infections will exceed the reported clinical cases by at least 10-fold.[17] The seroepidemiology of cholera depends on the specific but short-lasting vibriocidal antibody response, so long-term retrospective serosurveys like those done for viral pathogens cannot often be meaningfully conducted for cholera.

In the developed world, when a cholera case is detected, it may be useful to heighten local surveillance to detect other cases, and thereby establish whether transmission is ongoing. This is done by obtaining cultures from patients with severe diarrhea coming to medical attention and by testing convalescent sera from severe diarrheal cases seen at medical facilities in the preceding weeks.[15,18] If sewage collection is centralized, culturing gauze Moore swabs placed in the main sewer for 24 h can detect *V. cholerae* in areas drained by the sewer.[19] This is an economical way of maintaining surveillance on a population among whom cholera might be introduced.[15]

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organism. *Vibrio cholerae* is excreted in stools of the cholera patient and grows well on several standard bacteriologic media. Efficient isolation depends on the use of a selective medium that suppresses other bacterial species, while permitting *V. cholerae* to grow. Thiosulfate–citrate–bile salts–sucrose (TCBS) agar is the most widely used medium.[15] Alkaline peptone water (APW) is frequently used as an enrichment broth. [15]*Vibrio cholerae* produces large yellow colonies on this medium. The isolates are further characterized biochemically and tested with diagnostic antiserum for the presence of O1 antigen. The O1 strains will agglutinate in the O1 antiserum, producing a coagulated clump on a slide. In the same manner, O139 strains can be identified with O139 antiserum. Strains that agglutinate in neither O1 nor O139 antiserum may be non-O1, non-O139 strains of *V. cholerae* or other closely related vibrios (such as *Vibrio mimicus*). Definitive identification of such strains may require the services of a reference laboratory, but is not necessary if the only concern is cholera. Strains that agglutinate in O1 antiserum can be further characterized as serotype Inaba or Ogawa by using monovalent antisera.

For environmental or food specimens, in which the number of *V. cholerae* organisms may be much lower than in stools, an enrichment step is often necessary. Incubating the specimen in APW for 8 h takes advantage of the affinity *V. cholerae* has for alkaline environments; the broth is subsequently plated on TCBS.[15]

The O1 or O139 strains should be further characterized by whether or not they produce cholera toxin. This was originally measured by the ability of supernatant from a broth culture to induce the outpouring of fluid in the rabbit ileal loop, but is now detected by tissue culture assay, latex bead agglutination, enzyme-linked immunosorbent assay,[15]or PCR. [20]

Strains can be further characterized by biotype, of which there are two, classical and El Tor. However, because

classical biotype is restricted to coastal Bangladesh, the need for biotyping is minimal in most places.

Within biotype E1 Tor, subtypes can be defined by using traditional and molecular techniques (see Section 4). Subtyping is of value in epidemiological investigations to help determine the source of infecting strains.

Some more rapid presumptive diagnostic methods exist. The characteristic darting "shooting star" motility of vibrios can be observed by microscopy of a wet mount of fresh feces and halts immediately if O1 antiserum is added to the slide.[21] A commercially available diagnostic test based on colorimetric immunoassay detection of O1 antigen offers rapid presumptive diagnosis in the field without microscopy.[22,23] The efficiency of this test equals that of traditional microbiological methods; using the test provides greater speed of diagnosis at higher cost. A recent evaluation of this test and two other rapid diagnostic methods revealed that a dipstick assay using monoclonal antibodies in the detection of *V. cholerae* O1 and O139 lipopolysaccharides was highly sensitive in the hands of both field workers and laboratory technicians.[24] This feature, along with the fact that it does not require refrigeration, makes it a potentially useful tool in outbreak detection. It has recently been made available through the commercial sector at a cost of roughly US $1.

Rapid methods do not yield a living organism for confirmatory or antimicrobial resistance testing. Since a patient with severe diarrhea requires rehydration therapy whether or not cholera is confirmed, rapid tests should not affect management of the individual patient. Rapid diagnosis may prove most useful when it is linked to public health actions that slow the spread of an epidemic.

3.4.2. Serological and Immunologic Diagnostic Methods. The human immune response to infection with toxigenic *V. cholerae* O1 has been well characterized.[25] Within 10 days, vibriocidal antibodies appear in the serum, which, like viral neutralizing antibody, rapidly kill target *V. cholerae* O1. This response begins to decline by 1 month and then disappears after a year. There is no difference between antibodies evoked by Ogawa or Inaba strains, though in general, classical strains appear to evoke higher antibody responses than E1 Tor strains. The somewhat slower antitoxic response peaks by 21–28 days after exposure and remains elevated for several years.

Serodiagnosis of the single case depends on demonstration of a fourfold rise in vibriocidal titers between early and convalescent sera or a fourfold fall between convalescent and late convalescent sera.[15] Detecting antitoxin is suggestive evidence and is helpful when trying to distinguish between vaccine-induced and natural immunity (if the vaccine does not contain toxin subunits). However, similar antibodies appear after infection with *Escherichia coli* producing heat labile enterotoxin (ETEC LT). Methods for distinguishing the two responses exist and are applicable in population surveys, but they may not resolve the isolated case.[26]

The immune response to infection with toxigenic *V. cholerae* O139 is incompletely understood.[27] However, it appears that previous infection with O1 does not offer protection against O139.[28,29] A study comparing the immune response of patients infected with these two serogroups revealed that both serogroups can elicit a vibriocidal and cholera toxin-specific response. Similar to the response seen with toxigenic O1, patients infected with O139 had a cholera toxin-specific response and LPS-specific antibody response which peaked by day 7.[29]

4. Biological Characteristics of the Organism

Vibrio cholerae organisms are gram-negative, comma-shaped rods, adapted to life in brackish water. They are highly motile, with a long terminal flagellum that propels them through water, and they metabolize a wide variety of nutrients. They grow most rapidly at a pH that is neutral to somewhat basic, in moist environments with traces of salt and organic matter. This includes many foods, such as cooked grains, like rice or lentils, and cooked shellfish.[30] They are exquisitely susceptible to drying, sunlight, and acid, and die rapidly at pH of 4.0 or less. Humans are the only vertebrate host.

Serogroups of *V. cholerae* have been defined on the basis of their surface antigens. Like other gram-negative organisms, *V. cholerae* produce lipopolysaccharide O-antigen with a variety of terminal sugar groups that can be differentiated serologically. The flagellar antigens (H-antigens) do not vary within the serogroup and are not used for subtyping.[31] Only two serogroups, O1 and O139 are "choleragenic" and hence have the ability to cause epidemics. Both of these serogroups possess the cholera toxin, an important virulence determinant for *V. cholerae*. Within the O1 or O139 serogroups, nontoxigenic strains occasionally occur that may cause diarrhea but do not cause cholera. Cholera toxin is a protein enterotoxin formed from an active A subunit of 240 amino acids, held within a pentamer ring of five B-subunits, each of which has 103 amino acids.[32] The organism varies the amount of toxin produced depending on its immediate environment.[33]

Within the O1 serogroup, two specific antigenic serotypes, called Ogawa and Inaba, have been defined. Serotype switching has been observed within a given strain, so that these are not stable markers.[13]

Toxigenic O1 strains can be separated into two biotypes: El Tor and classical. El Tor strains agglutinate chicken red cells and lyse sheep red cells, while classical strains do neither; the two biotypes can be separated by phage

susceptibility studies as well.[34] Classical strains caused global pandemics in the late 19th and early 20th centuries, but since then have been restricted to rural Bangladesh.[35] The El Tor biotype of *V. cholerae* O1 is named for a Hajj quarantine station in Egypt, where it was isolated from an asymptomatic pilgrim early in the 20th century. Its epidemic potential was debated until the beginning of the seventh pandemic in 1961. There are important epidemiological differences between the two biotypes. Compared to classical strains, El Tor strains are more likely to produce inapparent infections,[17] persist longer in the environment,[36] multiply more rapidly following inoculation into foods,[30] and evoke less complete immunity.[37]

Toxigenic *V. cholerae* O1 El Tor strains can persist in rivers and estuaries for long periods, indicating they have niches in nature that are independent of human fecal contamination. This persistence is best demonstrated for the unique strain found along the US Gulf Coast[38] and for that found in the saline rivers of northeastern Australia,[39] though other natural foci are likely to exist. The precise nature of the niche is unknown, though several clues point to an association with plankton. *Vibrio cholerae* O1 produces chitinase, an enzyme that dissolves chitin, the structural protein of invertebrate exoskeletons.[40] This suggests they may have a niche in the digestive tracts of marine life that eat crustacea, or even a role in the growth and molting pattern of some crustacea. They can attach directly to crustacea, particularly to zooplanktonic copepods, without harming them.[41] They may persist in association with certain algae.[42] A dormant phase, the so-called "viable but noncultivable state," has been hypothesized to describe the persistence of *V. cholerae* in the environment between epidemics.[43,44]Environmental factors, including temperature and salinity, may allow for the transition between the dormant and transmissible phase.[45]

Three other enterotoxins produced by *V. cholerae* O1 have been described although their role in human disease is uncertain. These are zona occludens toxin (ZOT),[46] accessory cholera enterotoxin (ACE),[47]and WO7 toxin.[48]It appears that the ACE results in the secretion of Cl^- and HCO_3^-, mediated by Ca^{2+} as a second messenger.[49] The ZOT induces a diarrheal response by reversibly altering the structure of the tight junctions, and hence increasing mucosal permeability.[50]Although the mechanism of the WO7 toxin is uncertain, it does agglutinate rabbit erythrocytes and produce fluid accumulation in the rabbit ileum.[48]

In addition to cholera toxin, a pilus colonization factor, toxin coregulated pilus (TCP), is necessary to cause disease.[51] While cholera toxin is encoded by a lysogenic filamentous bacteriophage, CTX,[52] the mechanism of TCP acquisition is still uncertain.

Vibrio cholerae O139, first detected during outbreaks of cholera-like illness in 1992 in India,[53] appears to derive from O1 strains of biotype El Tor. Although it shares biological traits, including the production of cholera toxin, with *V. cholerae* O1, *V. cholerae* O139 does not agglutinate with O1 antisera[53], and possesses both a capsule and a distinct lipopolysaccharide.[54] A portion of the genome coding for O-antigen synthesis has been deleted from otherwise typical El Tor O1 strains, and genes have been inserted that code for a different antigen expressed as a polysaccharide capsule.[55]This evolutionary event appears to be mediated by horizontal gene transfer via a bacteriophage.[56] The noted differences between O1 and O139 may explain the lack of cross-protection.

Molecular subtyping methods have revealed a surprising diversity of subtypes within epidemic strains. Even the highly conservative methods of multienzyme electrophoresis identify four main enzyme types (ET) within El Tor strains, representing the Gulf Coast, Australian, seventh pandemic, and Latin American strains; the same method showed that at least three subgroups could be distinguished in the classical biotype.[57] Ribotyping identified nine ribotypes (RT) among 176 toxigenic El Tor strains tested.[58] With pulsed field gel electrophoresis (PFGE), 36 patterns were identified among 142 toxigenic El Tor strains tested, offering a fine-grain subtyping that is useful for epidemiological investigations.[59] The Latin American epidemic strain, introduced in Peru in 1991, remained relatively uniform,[60] although some minor genetic variations emerged in strains from Lima during the first few years of the epidemic.[61] The diversity of epidemic strains in Latin America increased as the original one was joined by other strains, likely to represent separate introductions.[60,62,63]

These findings are consistent with the seventh pandemic in the rest of the world, which is caused by an overlapping patchwork of strains. Using the same methods, strains associated with the O139 epidemic can be divided into at least two ribotypes and four PGFE types from the beginning, indicating that the epidemic of O139 infections is caused by more than one "epidemic strain."[64]

The genes coding for cholera toxin B-subunit also show diversity, and at least three subtypes exist.[65] All toxin types appear to have the same biological activity. Curiously, the Gulf Coast El Tor strains have the same B-subunit type as otherwise unrelated classical strains, suggesting that toxin genes have transferred horizontally between unrelated bacteria.

Vibrio cholerae of other non-O1 serogroups can sometimes cause human illness, including diarrheal illness and lethal invasive infections, but have not spread in epidemic form. Although they are often isolated from the aquatic

environment, most non-O1 *V. cholerae* serogroups are of little importance to public health, and care is needed in the interpretation of reports that do not specify the serogroup under discussion. Recently, isolates of *V. cholerae* O141 that possess cholera toxin and are capable of causing severe cholera-like illness have been described in sporadic cases in the United States and elsewhere.[66, 67] To date, no outbreaks or epidemics have been attributed to this strain.

The complete genome of *V. cholerae*has been recently sequenced. It consists of two circular chromosomes, the larger chromosome I, comprising some 2.96 million bp, contains most of the essential genes as well as loci determining virulence.[68] The toxin-associated genes reside within a lysogenized filamentous phage, consistent with the hypothesis that they originated from another organism via horizontal transfer.[52] Chromosome II, which consists of roughly 1.07 Mb, may have been acquired as a plasmid or by excision from a single large genome and harbors unique copies of critical genes, including a large cluster of integrons, mobile DNA elements capable of gene acquisition.[68]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

In 2007, 53 countries reported cholera to the World Health Organization.[8] The 117, 963 cases and 4,031 deaths reported in 2007 are only a small fraction of the true global total and are largely driven by Africa, which reported 116, 583 cases, 94% of the total, including large outbreaks in multiple countries. A recent review of WHO data on reported cholera cases and deaths revealed that the incidence of indigenous cholera in sub-Saharan Africa was 95 times higher than the reported incidence in Asia and 16,600 times higher than the reported incidence in Latin America in 2005.[69] Over 60% of all cases reported globally were reported from just four countries in Africa (Angola, Democratic Republic of Congo, Ethiopia, and Somalia). In 2007, the reported case fatality rate in Africa was 2.4%—seven times greater than the rate reported in Asia (0.33%).[8] These differences may reflect differences in reporting (for example, no deaths were reported from Bangladesh), pre-existing immunity, host factors (such as blood group—see Section 5.7), and the delivery of medical care to cholera patients. There is no evidence to suggest a change in the virulence of the organism.

The incidence of cholera depends on the presence of the causative organism, the opportunities available for its transmission, and the population immunity. Where sanitation is poor and crowded and previously uninfected populations are at risk, cholera incidence can be extremely high. However, even then most infections are asymptomatic. It has been estimated that 2% of infections are severe, 5% are moderate, and 18% have mild symptoms; up to 75% of infections are inapparent.[17] In the first year of the epidemic in Peru, 1.5% of the population was reported to have clinical cholera,[70] and by early 1995, this had risen to 3%.[71] It is likely that most Peruvians were infected. In remote African villages affected by outbreaks, incidence was 1.5 per 100.[72] In Bangladesh, where infection is likely to be nearly universal and repeated, silent infections maintain a relatively high level of immunity in the population. Nonetheless, the annual incidence of cholera requiring hospitalization ranges from 0.2 to 5 per 100.[73]

Different strains of *V. cholerae* may compete with each other. In Bangladesh, where E1 Tor and classical biotypes of *V. cholerae* coexist, the relative prevalence of these strains varies from year to year unpredictably, possibly reflecting shifts in the underlying ecology.[35,73] In 1992, *V. cholerae* O139 emerged in Bangladesh, caused epidemic disease, and transiently displaced O1 as the dominant strain.[74] While O139 continued to dominate in the southern coast region of Bangladesh, by 1994, a new clone of O1 was again seen in the northern and central areas.[75] Between 1996 and 2002, cholera was primarily attributed to O1. In early 2002, O139 again emerged as the epidemic strain.[76] Surveillance studies indicate that multiple ribotypes of O139 are now present. If genetic changes provide an evolutionary advantage, more widespread dissemination of the O139 strain may yet occur.[76]

5.2. Epidemic Behavior and Contagiousness

Cholera can appear as a single isolated case, as focal outbreaks, or in large epidemics. In the developed world, even sporadic cases are rare and are usually the result of travel to the developing world or of consumption of undercooked shellfish harvested from a natural reservoir, as with other *Vibrio* infections. When focal outbreaks occur, they are due to a common food source, and secondary transmission is rare.

In the developing world, where sustained transmission is possible, a single case or a focal outbreak can lead to larger epidemics. In small closed groups, such as a village or barracks, transmission halts after several weeks, when there are no more susceptible individuals or when effective control measures are introduced. In larger populations, an epidemic can persist for many months, slowing in the cooler season and then recurring the following year. In some areas, cholera then disappears; in others, it recurs regularly for many years, presumably because the organism finds an environmental niche. A long-term carrier state has been described

in humans, but is extremely rare and does not play a defined role in the persistence of the organism.[77]

Throughout the 19th and early 20th centuries, cholera rarely lasted more than a few years in one location. However, since the beginning of the seventh pandemic, cholera has tended to persist or recur once it was introduced. Cholera remains a public health threat in Africa now, nearly four decades after it entered that continent in 1970. Indeed, nearly 94% of the reported cases of cholera in 2007 were from Africa, a percentage consistent over the past decade.[8] While it persisted for some years in several areas of Latin America since its arrival 1991, this region did not report any cases of cholera in 2007.[8] This tendency to persist may be related to the specific properties of E1 Tor strains. It may also be related to the far greater density and mobility of human populations now compared with 100 years ago. It suggests that in the absence of major improvements in water and food quality and sanitation, or widespread implementation of effective immunization campaigns, cholera will remain an important global public health threat for many years to come.

Because a large inoculum is required to produce disease, cholera is rarely transmitted directly from one person to another without the intervening contamination of food or water. Caregivers themselves are very unlikely to become infected. Episodes of nosocomial transmission are likely to be the result of contaminated water or infant formula, not direct person-to-person spread.[78] Cholera often spreads within households in the developing world. On the Micronesian island of Chuuk (Truk), this was particularly likely if the first case in the household occurred in the food preparer.[79] In Kolkata, secondary cases were more likely if the household stored drinking water in a large-mouthed container, where it was easily contaminated.[80] Cholera outbreaks have occurred among persons attending funerals of cholera victims. This is less likely to be due to simple contact with the cadaver, and more likely to be due to consuming foods and beverages at a funeral meal that were prepared by bereaved family members after they prepared the corpse for burial.[81]A case–control study to assess the role of disinfection and funeral activities was conducted during a 1994 outbreak in Guinea-Bissau. This study revealed the protective effect of disinfection, finding an association between disease and eating at a funeral with a non-disinfected corpse as well as touching a non-disinfected corpse.[82]

When an epidemic occurs, it is sometimes possible to determine the original source of the infection. This may be a traveler who arrives while still incubating the infection, someone bringing contaminated foodstuffs with them, or even the body of a victim being brought back to an ancestral village for burial. It is rare to identify any measure that could have prevented the introduction. *Vibrio cholerae* can also be introduced via ocean freighters, which take on ballast water in one harbor and discharge it in the next, contaminating shellfish beds near harbors in the process.[83] To decrease this risk, freighters are now required by the International Maritime Organization to exchange their ballast water while at sea. The source of the unique strain that appeared in Latin America in 1991 remains unknown.[70] Several theories regarding its source have been postulated, including freighters with passengers from cholera endemic areas,[70] dissemination by cargo ship ballast,[84] and phage-mediated virulence gene transfer.[85] There has also been speculation that environmental changes associated with the El Nino phenomenon in 1991 could have fueled increased concentration of vibrios in the coastal waters.[86] Throughout the world, the tides of humanity that flow from place to place can introduce cholera as long as it persists anywhere.

5.3. Geographic Distribution

The seventh cholera pandemic has affected most of the developing world (Figure 2). Developing countries with higher levels of sanitation, such as Chile, Argentina, and Costa Rica, tended to have relatively low attack rates, and even in countries affected by epidemics, areas with better sanitation were less likely to be affected. In Mexico, cholera displayed a north–south incidence gradient, with the highest incidence in the poorest southern states.[70]

Unique strains of *V. cholerae* O1, biotype E1 Tor, persist in environmental reservoirs along the US Gulf Coast and in rivers of northeastern Australia. Strains of *V. cholerae* O1, classical biotype, are now limited to coastal Bangladesh, while E1 Tor biotype strains are found farther inland in the same country, suggesting that the two differ in their ecological requirements.[35]

Spread of the epidemic strain, *V. cholerae* O139 Bengal, has so far remained restricted to Asia (Figure 1), but could in the future reach Africa and other parts of the developing world, potentially causing the eighth pandemic.

5.4. Temporal Distribution

Cholera is sharply seasonal, becoming rare or undetectable in cooler months. It is not known what determines this seasonality or how the organism survives from one season to the next, however, recent data suggest that cholera phages may be responsible. The presence of phages were shown to be inversely related to the presence of *V. cholerae* strains found in the environment and to the number of reported cases.[87] Further, the ability of vibrios to become dormant, in the viable but non-culturable phase, in response to environmental factors may allow for their persistence. In the Latin American epidemic, cholera

increased sharply in the early summer and declined slowly in the fall. The calendar month affected varies with the hemisphere, peaking in July through September in the Northern Hemisphere and from January through March in the Southern Hemisphere.[70] Along the US Gulf Coast, the same seasonal pattern prevails.[88] In Asia, the seasonality tends to be predictable in a given location, but it is not uniform across the continent. Attempts to relate seasonality to rain, monsoon, or water temperature are inconsistent. In the 1950s, the peak season for cholera in Kolkata was May and June, just before the monsoons, while in Chennai, farther south on the same coast of India, it was August and September at the end of the monsoons.[1] In rural Bangladesh, the seasonality of E1 Tor and classical infections differ: the peak for E1 Tor is October–November, and for classical biotype is in December–January.[73] The seasonality may be related to the presence of algal blooms.[89] Whether this association is related to the environmental factors which favor algal bloom growth or their disintegration is unknown. One hypothesis suggests that the nutrients released at the end of algal blooms may favorably affect the growth of *V. cholerae*.[90]

Year-to-year variation in incidence can be substantial and may also reflect shifts in underlying ecology (Figure 4). In British Colonial India, it was noted that the year following a year of drought had a higher incidence of cholera.[91] Along the Gulf Coast, the years with highest incidence also happened to be years of drought, when saltwater incursions moved further inland than usual. No general predictive rule is known.

5.5. Age

When cholera affects a population that has no preexisting immunity, the age distribution of cholera patients reflects exposure to infection. In Piura, Peru, where the town water and street-vended beverages made with ices were identified sources, nearly all ages were equally exposed. Incidence in the first 3 weeks of the 1991 epidemic was 1.3% among those aged 5–14 years and 1.8% among those over 15 years of age.[92] In Italian outbreaks of 1973, transmission occurred through contaminated raw shellfish that were usually eaten by adults, so the median age of patients was 52 years.[93] Because gastric acidity declines with advancing age, the elderly are often more likely to have severe illness than younger persons. In Mexico, in 1991, the incidence among those over age 65 years was 7.1 per 100,000, compared with 2.5 among persons aged 15–24 years.[70]

In Bangladesh, where many adults have natural immunity that may be boosted by repeated exposure, cholera caused by *V. cholerae* O1 is more typically an illness of children, though cases in adults are not rare.[73] Breastfeeding infants are protected because they are not exposed to contaminated water and food and also because of passive protection of antibodies in maternal milk.[94] When epidemic *V. cholerae* O139 infections appeared in the Bengal area, pre-existing immunity to O1 strains had little effect, and all ages were affected.[95]

5.6. Sex

The gender of the patient does not affect the outcome of infection or the rate of infection, given equal exposure.

5.7. Race

Cholera and race are not specifically linked. However, severe manifestations of cholera are strongly associated with blood group O, the frequency of which varies among races. Persons of blood group O are more likely to have severe cholera than persons of other blood groups. In Bangladesh, where blood group O is relatively uncommon, most adults hospitalized with cholera were of blood group O.[96] It has been speculated that the reason that blood group O is so rare in this population is that cholera itself provided a selective pressure over many centuries. In the Americas, most native peoples are blood group O, so this blood group is extremely common throughout Latin America. In Peru, persons with blood group O were no more likely to be infected with *V. cholerae* than others, but if infected they were ninefold more likely to develop life-threatening diarrhea than persons with other blood groups.[97] The reason for this strong association is unknown.

5.8. Occupation

Few occupational associations are apparent in cholera epidemics or outbreaks. Fishermen and harbor workers may be the first to be affected. The first case of cholera on the US Gulf Coast occurred in a shrimp fisherman in 1973.[98]

Notably absent are cases among health staff caring for cholera patients, among microbiologists, and among undertakers preparing the bodies of cholera victims for burial. The extreme rarity of such cases underscores the ease with which simple hygienic precautions prevent cholera transmission.

5.9. Occurrence in Different Settings

Cholera epidemics occur when breaks in sanitation lead to contamination of water and food consumed by many people. The most characteristic setting is the large periurban slum, where people newly arrived from rural areas outstrip the availability of basic urban services (see Section 6). Temporary gatherings have also been recurrent sites of cholera outbreaks, including religious pilgrimages and

refugee encampments. Historically, the Islamic Hajj has played an important role in disseminating pandemics, as have the great religious migrations of India.[1] Cholera has not complicated the Hajj in recent years, perhaps because of efforts to provide better sanitation during the pilgrimage; pilgrimages in Bangladesh may have accelerated the spread of *V. cholerae* O139. Concern for the spread of cholera at traditional Andean pilgrimage sites, such as Copacabana on Lake Titicaca, prompted authorities to limit the size of the pilgrim population and to match it with latrine and potable water facilities; to date, pilgrimages have not been a major source of spread of cholera in Latin America.

Refugee camps in Asia and Africa have experienced large epidemics of cholera. These epidemics are most severe when the camps are just being established or are overwhelmed with a new influx of people. The sudden migration of 700,000 Rwandan refugees to bare lava fields near Goma, Zaire in the summer of 1994, where there were no latrines or wells, led to a catastrophic cholera and dysentery epidemic that killed 5% of the population in the first 3 weeks of the camp.[99] Cholera had recurred in the region for years, suggesting there is an environmental reservoir in Lake Kivu, the water of which was drunk without chlorination. In more organized camps of Mozambican refugees in Malawi, epidemic cholera was recurrent, though with better medical care the mortality was lower. Cases disproportionately affected children and were most common among the most recent arrivals to the camps, who lacked cooking gear, fuel, and water storage pots as well as those who drank river water.[100] However, cholera is not inevitable among refugees.[101] Cholera outbreaks were rare among Vietnamese refugees in Thailand and were easily controlled once the source was determined.[102]

Epidemic cholera has rarely followed natural disasters, except in the Bengal basin. In Bangladesh, epidemics of "severe diarrheal disease" occur after typhoons flood coastal regions, in areas that would have had many cholera cases even without unusual flooding. In 1998, a large epidemic, with greater than 16,500 reported cases and 276 deaths, occurred after severe floods in West Bengal, an area with endemic disease.[103] On December 26, 2004, a devastating tsunami, which originated in the Indian Ocean, killed an estimated 275,000 people in affected countries around that ocean. Although this event raised concern for the spread of a host of infectious diseases, there has been no confirmed cholera cases reported to WHO related to this tragedy.[104]. Similarly, although sporadic cases of wound and gastrointestinal *Vibrio*infections were reported following Hurricane Katrina's devastation of the US Gulf Coast, epidemic cholera did not occur.[105]Surveillance did identify two cases of toxigenic *V. cholerae* O1 reported in New Orleans in the weeks after Hurricane Katrina.[106] Both cases were associated with consumption of inadequately cooked Gulf Coast shellfish.

5.10. Socioeconomic Factors

In most epidemics, cholera is associated with extreme poverty. In the developing world today, just as in the industrializing cities of Europe over 150 years ago, better housing usually implies better water and safer food supplies. Behaviors that protect against cholera cost money: these include boiling water, storing it in special containers to protect it from recontamination, and heating food for each meal rather than eating cold leftovers.

5.11. Other Factors

Because vibrios are exquisitely sensitive to acid, the patient's gastric acid barrier is important. Gastric acid production is decreased by gastric surgery for ulcer disease, by antacid or antiulcer medication, and in the youngest infants and those of advanced age. This risk factor can be an important determinant of who gets ill. For example, in an Italian outbreak in 1973, 27% of patients had had gastric surgery.[93] The impact of infection with the gastric bacterium *Helicobacter pylori*, which can induce a state of hypochlorhydria, is less well understood but may be important (see Chapter 18). A cross-sectional study of the 1991 epidemic in Peru found that *V. cholerae* O1 was associated with infection with *H. pylori*. This association was more pronounced in children and yielded a greater adjusted odds ratio than blood group O.[107]

6. Mechanism and Routes of Transmission

Cholera follows ingestion of food or water contaminated with an infectious dose of the causative organisms. In epidemics, the source of contamination is usually the feces of infected humans. In sporadic cases associated with natural reservoirs, no fecal contamination need occur.

In the United States, the majority of cases are acquired outside of the country.[108] Locally acquired cholera is usually associated with eating undercooked seafood harvested on the Gulf Coast.[98] One outbreak on a Texas oil platform in the Gulf of Mexico followed contamination of rice with seawater, which itself was contaminated by the platform's sewage line; it is presumed that fecal contamination played a role.[109] Other cases have been related to foods that have been cross-contaminated[108] documented by one outbreak

Table 2. Precautions for Travelers to Areas with Epidemic Cholera[a]

- Drink only water you have boiled or treated with chlorine or iodine. Other safe beverages include tea and coffee made from boiled water and carbonated, bottled beverages with no ice
- Eat only foods that have been thoroughly cooked and are still hot, or fruit that you have peeled yourself
- Avoid undercooked or raw fish or shellfish, including ceviche
- Make sure all vegetables are cooked—avoid salads
- Avoid foods and beverages from street vendors
- Do not bring perishable seafood back to the United States
- A simple rule of thumb is "Boil it, cook it, peel it, or forget it"

[a]From CDC public information brochure, "Cholera Prevention," published May 1992. Available at the Web site http://www.cdc.gov/ncidod/dbmd/diseaseinfo/cholera_g.htm

which followed the contamination of a melon by an asymptomatic food preparer.(110) Additional cases are brought from countries where cholera epidemics were occurring, e.g., two outbreaks were traced to crabs from Ecuador and an outbreak was related to fresh frozen coconut milk imported from Thailand.(98, 111)

Travel-associated cases typically occur among persons who are visiting friends or relatives in their own countries of origin in the developing world. These travelers tend to be exposed to the same risks of cholera as the developing country population. Cases among more typical tourists or business travelers are extremely rare. The overall infection rate is remarkably low, approximately 1 per million air travelers to affected countries.(88) Simple precautions should prevent most of these cases (Table 2).

In the developing world, transmission of cholera has been documented through a variety of water and foodborne mechanisms. During epidemics, infections can be spread by more than one mechanism, and the dominant source of infection may change. In an epidemic on the Gilbert Islands, the initial cases were caused by consumption of partially dried salted fish, but a month later illness was associated with eating a variety of raw clams and shrimp as well as salted fish.(112) In 1974, during a cholera epidemic in Portugal, an initial wave of cases associated with eating raw shellfish was followed by a sudden surge when a popular brand of bottled water became contaminated, thereby infecting those who drank the water precisely to avoid cholera.(113,114) In eight collaborative investigations into the mechanisms of transmission of cholera in Latin America, multiple mechanisms of transmission were identified, including different mechanisms in different cities in the same country (Table 3).

In Latin America, seven of eight collaborative investigations identified waterborne transmission as a major element of risk for epidemic cholera (Table 3). Waterborne transmission occurs when feces from an infected person contaminate a stream, lake, or shallow well used for drinking water; when municipal water supplies draw from a contaminated source; or when water that was initially clean is contaminated during or after distribution. Water pipes are often laid in the same trench as sewer pipes. If water pressure drops, because of an intermittent water or power supply, then the low pressure in the water pipes can draw sewage into the water through minute inadvertent cross-connections. In this

Table 3. Mechanisms of Transmission of Epidemic Cholera in Latin America Documented in Epidemiological Investigations, 1991–1993

	Peru	Peru	Peru	Ecuador	El Salvador	Bolivia	Brazil	Guatema
Location	Trujillo	Piura	Iquitos	Guayaquil		Saipina	Fortaleza	Guatemala City
Setting	Urban	Urban	Urban	Urban	Rural	Rural	Rural	Urban
Reference	111	90	113	146	77	78	*a*	115
Transmission mechanism								
Waterborne								
Municipal water	+	+		+				
Surface water			+		+	+	+	
Putting hands in water vessel	+	+						
Street vendors								
Foods		+						+
Beverages		+		+				+
Ice/ices		+						+
Other foods								
Uncooked seafood				+				
Cooked seafood				+	+			
Leftover rice		+	+					+
Fruits/vegetables		+						

[a]CDC unpublished data.

way, a few cases of cholera that contaminate sewage can multiply rapidly into a citywide epidemic. Authorities who limit their monitoring to the initial quality of water going into the piping system at the wellhead may see no need for chlorination.

Because water is rarely available 24 h a day in many municipal systems and is often collected from shared public standpipes in the developing world, drinking water is usually stored in the home. If the vessel used to store water has a wide mouth and if water is removed by scooping it out by hand, stored water is likely to be further contaminated. Increasing water contamination was measured in Trujillo, Peru in 1991: fecal coliform counts were 1 per 100 ml in water collected at the wellhead, 2 per 100 ml at public taps, and 20 per 100 ml in water stored in the home.[115] This phenomenon was further clarified in a systematic review of the relationship between water quality at point of use and both cholera and general diarrhea. Water quality was measured by coliform counts and this analysis found that while interventions involving the treatment and storage of water were not protective against general diarrheal diseases, such measures did clearly reduce the burden of illness attributed to cholera.[116] Protective practices include using a small-mouthed vessel to store water, pouring water from the vessel rather than scooping it out, treating water in the home with chlorine or by boiling, or adding citrus juice to the water to acidify it.[117]

Foodborne transmission can also occur, particularly through moist grains such as rice or millet and by seafood. Transmission by foods other than seafood was identified in four of eight collaborative investigations in Latin America (Table 3). When leftover moist grains are held at ambient temperature, a few vibrios can grow to a large dose by the next meal. The risk depends on the sauces that accompany the grain. In Guinea, cholera was associated with the use of a peanut sauce with neutral pH, while an acidic tomato sauce was highly protective.[118] In Mali, cholera was transmitted by millet, a grain that usually would have been made with acidic fermented goats' milk. Because of drought, the goats were dry, and the millet permitted survival of vibrios.[72] Transmission has occurred from fruits and vegetables, which have been documented to have been irrigated with fresh sewage or splashed with sewage on the way to market.[117] *Vibrio cholerae* can be transmitted in street-vended foods and beverages, which are often prepared in unhygienic ways and then held for many hours at ambient temperatures. In Piura, Peru, street beverages made with contaminated ice were an important source of cholera.[92] In Guatemala, frozen fruit drinks were the dominant source.[63]In Zambia, the consumption of raw vegetables was associated with cholera. Interestingly, these investigators found that the consumption of kapenta, a dried sardine-like fish, and the presence of soap in the home, were protective.[119]

Seafood can be an important source of cholera in the developing world when shellfish are harvested from heavily contaminated waters and processed with little regard to sanitation. Transmission of cholera through seafood occurred in two of eight collaborative investigations in Latin America (Table 3). Vibrios can easily survive light cooking and then grow to an infectious dose if the seafood is held for several hours at ambient temperature. In Guinea-Bissau, cholera was associated with eating small crabs, particularly as leftovers.[81] In Peru, ice used to ship fish was not made from chlorinated water in 1991, because "it was not for human consumption," although the fish were typically eaten without cooking.[92]

In the urban developing world, it is typical to find multiple layers of transmission occurring simultaneously. For example, in Manila, cholera was associated with municipal water contaminated at the periphery of the distribution system, and with street-vended foods.[120] One common implicated street food was a mussel soup made of shellfish harvested from aquaculture in a contaminated bay.

Food served on international flights has twice been implicated as the cause of cholera outbreaks. One outbreak occurred in 1972, on a flight from Great Britain to Australia, and a larger outbreak occurred in 1992, affecting 75 passengers, on a flight from Argentina to Los Angeles, via Peru.[121,122] Following the latter, in which cold seafood salad served enroute from Peru was epidemiologically associated with infection, the Centers for Disease Control and Prevention (CDC) advised the International Air Transport Association to stock oral rehydration solutions on international flights and to avoid serving cold salads or hors d'oeuvres prepared in cities with cholera epidemics.[123]

Transmission of *V. cholerae* O139 has not been well studied, but is likely to be similar to that of O1. Repeated isolation from water suggest that waterborne transmission is likely. A case–control study, which included cases of both O1 and O139, demonstrated similar modes of transmission among these two serogroups, including the consumption of untreated water and raw seafood.[124]A retrospective cohort study to assess the source of a *V. cholerae* O139 outbreak on a cruise ship found that illness was associated with eating yellow rice at a buffet in Thailand.[125]

7. Pathogenesis and Immunity

The severity of symptoms varies with the dose of bacteria ingested, the effect of gastric acid, and the patient's blood type (see Sections 5.7 and 5.11). In volunteer feeding trials,

ingesting 10^6 organism with bicarbonate buffer or mixed in food regularly results in severe diarrheal illness, whereas doses of 10^9 or more are needed if the vibrios are consumed in water without buffering gastric acidity.[126] Lower doses may result in severe illness if the bacteria are protected by the buffering action of food or if little gastric acid is present for other reasons.

Following ingestion, the bacteria pass through the stomach and reach the small intestine. In that alkaline and bile-rich environment, they attach to mucosal cells and produce cholera toxin. Toxin production is regulated by sensors that react to the surrounding environment. Once toxin is produced, the toxin B-subunits bind to specific ganglioside GM_1 receptors on the surface of intestinal mucosal cells.[127] A portion of the A subunit enters the cell and activates adenylate cyclase, producing high levels of cyclic AMP in the cell and active pumping of chloride ion into the intestinal lumen. As Cl^- is pumped into the intestine, potassium, bicarbonate, and water follow passively. The intestines fill with an alkaline, salty fluid, which is an ideal growth medium for *V. cholerae* which do not invade, and remain within the gut. Diarrhea begins when the colon cannot absorb the fluid fast enough and can reach 1 L/h. For a 70-kg adult, this represents a loss of 10% of body weight in 7 h, unless fluids and electrolytes are replaced. Fluid loss leads to decreased blood pressure, and finally circulatory collapse. Loss of bicarbonate leads to a profound metabolic acidosis, with blood pH below 7.0. Loss of potassium leads to neuromuscular malfunction, painful muscle cramps, and ultimately to cardiac arrhythmia, which may be the terminal event.

Rapid replacement of lost fluid and electrolytes is the critical lifesaving treatment.[128] For the most severe cases, this is done with intravenous polyelectrolyte fluids such as Ringer's lactate. Oral rehydration solution (ORS), a carefully defined mixture of carbohydrate and electrolytes, should be used to replace losses in patients who are able to drink, saving lives for pennies. This low-cost treatment is widely available throughout the developing world as WHO ORS or UNICEF Salts and represents a major breakthrough in the treatment of cholera and other dehydrating diarrheal diseases. Standard packets of either glucose-based or rice-based solutions are mixed with a standard volume of potable water and drunk to prevent or correct volume depletion. The presence of the proper amount of carbohydrate drives absorption of the electrolytes by the bowel mucosa. The optimal composition of ORS has been a matter of recent debate. Rice-based ORS have been shown to result in a greater reduction of stool output than glucose preparations.[129] In an attempt to improve the efficacy of ORS, reduced osmolarity solutions were further studied. A systematic review of ORS among a pediatric population found that the reduced osmolarity solution was associated with reduction in stool output, vomiting, and intravenous fluid use.[130] Based in part on these findings, the WHO, in 2002, advocated for reducing the osmolarity of the solution from 311 to 245 mOsm/L. Although the reduced osmolarity solution, in the above study, did not result in a significantly different serum sodium level, there has been concern regarding the possibility of ORS-induced hyponatremia and subsequent sequela.[131]

An additional concern regarding ORS is the water used to make the solution. As a result of being prepared from unclean water or of improper handling, ORS is often contaminated by enteropathogenic bacteria.[132]Disinfecting the water used to prepare ORS with bleach and storing the solution in narrow-mouth vessels was found to prevent bacterial contamination.[133]

Antibiotic treatment, for example, with tetracycline or trimethoprim–sulfamethoxazole, is a useful adjunct to fluid therapy, as it decreases fluid losses and hastens recovery, but it is not sufficient treatment by itself. For those with a contraindication (e.g., resistant strains or allergy) to the above antibiotics, the use of ciprofloxacin is an alternative.[134,135] A recent study found that a single dose of ciprofloxacin was as effective as a 3-day regimen of erythromycin among pediatric population.[136]

The diarrhea decreases after a few days of vigorous fluid therapy, and the patient recovers as soon as the fluid and electrolyte deficits are corrected. The diarrhea stops even though the intestines are still filled with toxigenic *V. cholerae*. Following infection, the patient is resistant to subsequent challenges with *V. cholerae* O1, but this protection is less than perfect and may not last for more than a few years. Infection with classical strains provides nearly complete protection against subsequent infection with any *V. cholerae* O1, while infection with E1 Tor strains provides only 60–70% protection to subsequent infection with either biotype.[37] This less solid immunity may help explain why the seventh pandemic has lasted so much longer than pandemics five and six. This is also the reason that vaccines are developed from classical strains, even though they are intended to protect against E1 Tor. Infection with O1 strains appears to provide no subsequent protection to infection with *V. cholerae* O139, although both produce the same toxin. This suggests that antitoxic immunity plays little role in the protective response.

8. Patterns of Host Response

8.1. Clinical Features

The clinical features of cholera are the result of a profound loss of intravascular fluids and electrolytes. The illness begins as a watery diarrhea, accompanied by abdominal

cramps, that typically starts 24–72 h after ingestion of the organisms. The diarrhea becomes voluminous and in severe cases continuous. The feces are like clouded water, with flecks of mucus, and are inoffensive smelling. They have been described since Osler's time as "rice water" stools, but could better be described as looking like thin chicken rice broth. As electrolyte disturbances become more pronounced, acidosis leads to vomiting and hypokalemia produces agonizing leg cramps, particularly in older patients. The signs and symptoms of dehydration are pronounced once fluid losses exceed 5% of body weight: dry mouth, absence of tears, increasing thirst, and loss of skin turgor, leading to the so-called "washer-woman's hands." As fluid losses reach 10% of body weight, the blood pressure drops and the patient becomes obtunded, nearing death from vascular collapse. Fever is rarely pronounced, and the patient's sensorium remains clear until the very end. The disease is reversible up to the moment of death, if fluid and electrolyte losses can be vigorously replaced. Transient renal failure may follow rehydration. Cholera can be particularly severe in the pregnant woman, and loss of the fetus is likely if infection occurs in the third trimester.[137] No increase in severity has been described among persons infected with human immunodeficiency virus (HIV).

Clinical manifestations of infection with *V. cholerae* O139 Bengal are similar to those of *V. cholerae* O1. However, several episodes of bacteremia have been documented with *V. cholerae* O139, as well as a case of subsequent reactive arthropathy.[124] It is possible that this organism is more invasive than *V. cholerae* O1, perhaps because of the capsule present in O139 strains.

8.2. Diagnosis

Diagnosis is made by isolation and identification of the causative organism or by demonstrating a diagnostic increase or decrease in the antibody response. In the outbreak setting, persons with similar illnesses may be presumed to have cholera once the etiology of the illness is established for a representative sample.

In the United States, cholera should be suspected and appropriate stool cultures requested from anyone with severe diarrheal illness who recently traveled to the developing world or who has ingested raw shellfish or undercooked crab.

In the developing world, in the setting of a known or threatened epidemic, presumptive diagnosis is made on clinical grounds. The report of two or more adults in the same place with severe watery diarrhea and vomiting or the report of an adult dying of watery diarrhea may be presumed to be cholera until proven otherwise. Immediate action should be taken to bring adequate supplies for treatment, prevention, and diagnosis to that location and to warn surrounding villages of the possibility of cholera.[11] A small number of rectal swabs from persons with fresh diarrheal cases should be collected and transported in Cary–Blair transport medium to a competent laboratory.

9. Control and Prevention

9.1. General Concepts

Toxigenic *V. cholerae* is unlikely to be eradicated, as it is likely to persist indefinitely in environmental reservoirs. Crowded urban populations in the developing world provide the setting for future epidemics of cholera when the organism is introduced, and increasing international mobility makes such introductions likely. As long as the organism persists anywhere, the potential for cholera exists wherever conditions are ripe for sustained transmission.

However, epidemic cholera can be prevented. Avenues of sustained transmission have been closed already in many parts of the world. Between 1961 and 1991, 56 travel-associated cases were reported in the continental United States without subsequent spread.[57] Between 1995 and 2000, 61 cases of cholera, 37 (61%) of which were acquired abroad, were reported in the United States.[108] Again, with the possible exception of a single case, subsequent spread did not occur.[110] In the United States and other industrialized nations, when a case of cholera occurs, prompt public health action is still warranted to determine the source of infection and to be sure that the feces are properly disposed of. Routine sanitation is adequate to prevent further transmission in most areas, but for some populations, including the homeless, the "colonias" along the Mexican border, and a few Native American reservations, the potential for further transmission still exists. Most populations on the North American and European continents are protected by the engineering triumphs of the "Sanitary Revolution," i.e., safe water and sewage treatment systems. Similar efforts now beginning could control cholera by the early 21st century in many rapidly developing countries of Asia and Latin America.[7]

In the rest of the developing world, where industrialization is slower, cholera prevention is an increasing challenge. A targeted health education effort may prevent illness and death by stressing the need for careful water and food handling and the need to seek medical care promptly for diarrheal illness. Emergency control measures such as boiling water are difficult to sustain. Large-scale engineering solutions are too costly to build and maintain, and there is a

critical need for simpler alternatives. Some promising strategies are being developed and tested. For example, because storage of drinking water in the home is nearly universal in the developing world, modification of the storage vessel to keep stored water from becoming contaminated is a simple and inexpensive strategy.[138,139] During an outbreak investigation on Ebeye Island, Republic of the Marshall Islands, researchers found that even chlorinated water could become contaminated, likely occurring during the transport and storage process, while using a tightly sealed container to haul water was protective.[140] A study in Kolkata found that cholera infection, as detected by stool culture, was less common among families using either "sorais", a narrow necked earthenware pitcher, or chlorine tablets, when compared to control families.[141] Sorais are designed such that the water must be poured out, rather than being scooped out by hand.

Drinking water can be disinfected routinely in the house at the "point of use".[139]In remote areas, a simple apparatus can produce a disinfectant chlorine solution by electrolysis of salt water, using solar power. With one such machine, a village of several thousand people can treat their own drinking water and make a net profit selling surplus disinfectant to their neighbors. Point of use chlorination effectively reduced the risk of cholera in Madagascar with a protective OR of 0.1.[142]Larger ventures to produce a water disinfectant product for routine use in the developing world are of potential commercial interest. Production of inexpensive chlorine-based disinfectants for household drinking water, linked to social marketing, is now occurring in 19 countries in the developing world.[143] Two studies have shown the benefit of solar disinfection of drinking water.[144,145] A recent study revealed that although there was no significant benefit of solar disinfection on cholera in adults, a clear benefit was seen in children under age 6 years. In this study, solar disinfection was performed by placing water in a clear, plastic bottle and leaving it on the roof, a procedure requiring minimal additional resources.[146] Closer attention to ice production in the developing world can guarantee that treated water is used to make ice, the cheapest of modern luxury goods.

Simple improvements in food preparation may also greatly reduce the risk of cholera. Acidification of sauces is routine for reasons of taste in many cultures and can be further promoted by public health authorities: tomatoes, tamarind, lemons, lime juice, and yogurt can all acidify foods sufficiently that *V. cholerae* cannot survive.[147,148]Even the use of powdered drink mixes, which contained citric acid and possibly ascorbic acid, was found to provide protection against cholera.[140] Foods can be made safer by thorough cooking, by heating or at least acidifying leftovers, and by avoiding raw seafood harvested from dubious waters. Particular attention to the problem of street vendor sanitation can include providing safer carts; promoting the use of clean water, soap, and disinfectants; training street vendors in the elements of food safety; and educating the consumers to look for vendors who are following simple and visible precautions.[149]

In many parts of the world, raw or undercooked seafood is a staple source of protein. Taking precautions to protect harvest waters and using clean water for processing and clean ice for shipping can help prevent seafood-borne cholera. However, as long as shellfish from warm waters are consumed raw, an irreducible minimum number of cases can still be expected because of the natural estuarine reservoirs of toxigenic *V. cholerae*.

When an epidemic of cholera appears, the first priority is to prevent death by assuring adequate treatment and by educating the population to seek treatment rapidly once a diarrheal episode begins. The success of efforts to control the epidemic depends on knowing the actual vehicles of transmission and having the tools available to block them. Because the vehicles may vary, swift epidemiological investigation, including a case–control study, may be critical to guide control efforts. In 1991, in Santiago, Chile, for example, case investigation suggested that cholera was being transmitted through use of fresh sewage to irrigate salad vegetables; halting this practice brought an abrupt end to this specific outbreak as well as decreasing typhoid fever incidence by 90%.[150] In 1974, in Portugal, successful control followed the identification of two sequential vehicles: contaminated raw shellfish and a spring used to make bottled water.[113,114] Throughout Latin America, recommendations to boil water and to avoid street-vended foods and raw seafood followed the identification of these routes early in the epidemic. Such emergency changes are not sustainable, and the persisting epidemic demands more fundamental approaches to prevention.[70]

9.2. Antibiotic and Chemotherapeutic Approaches to Chemoprophylaxis

Antimicrobial prophylaxis plays a limited role in the control of cholera. Prophylactic treatment of family members of cholera patients in the Philippines was shown to decrease the incidence of infection from 13% in the untreated group to 0% in family members taking 20 doses of tetracycline over 5 days.[151] In closed settings, such as prisons, ships, or small and isolated villages, the strategy has potential merit. However, chemoprophylaxis on any scale runs the risk of evoking antimicrobial resistance. Early in the West African epidemic, an extravagant "cordon sanitaire" strategy

included forced antimicrobial treatment of all who passed through roadblocks. This had no discernible effect on the progress of the epidemic, but resulted in widespread sulfa resistance in vibrios and avoidance of established roads. In Latin America, where the practice has been to avoid chemoprophylaxis, the organism remained generally susceptible in 1995, after a million cases had been reported. An instructive exception was in Guayaquil, Ecuador, where a vigorous campaign of family chemoprophylaxis in 1991 was associated with rapid emergence of multiple resistance, which forced the strategy to be abandoned.[152] In addition to the risk of causing the emergence of resistant strains and the substantial cost of antibiotics used in this way, mass chemoprophylaxis with sulfa or tetracyclines has been associated with severe adverse reactions to the drugs and with the appearance of folk belief that the antibiotic is a form of vaccine that confers permanent protection.

9.3. Immunization

In the past two decades, considerable progress has been made in the development of both live and killed oral cholera vaccines (Table 4).

Parenteral vaccines have historically not been favored given the low efficacy and high side-effect profile. A parenteral cholera vaccine licensed in the United States was withdrawn from production and sale in 2000. Although research has focused on the development of effective oral vaccines, as yet no vaccine is available that has broad public health applicability. Efforts to develop such a vaccine have been ongoing for more than a century as an inexpensive cholera vaccine that provided long-term protection against cholera would be an important public health tool.

Currently two vaccines are now available in some countries, although neither are licensed in the United States. These are an oral whole-cell killed *V. cholerae* O1 vaccine that includes a recombinant B-subunit of cholera toxoid (WC/rBS)[8], and an oral bivalent vaccine, currently available only in Vietnam, consisting of a mixture of heat and formalin killed O1 and O139 *V. cholerae* strains.[153] A live, attenuated genetically modified vaccine, CVD 103-HgR,[154,155] previously marketed as Mutachol, has recently been discontinued.

When WC/rBS was tested extensively in a field trial in Bangladesh in a location where both E1 Tor and classical strains were present, it had a protective efficacy in the first year of the trial of 62%.[156] Efficacy waned after 2 years, and was substantially lower among persons of blood group O,[157] among children, and against infection with E1 Tor biotype as opposed to classical biotype.[158] In order to assess the protective effect on herd immunity, data from the above studies were reanalyzed. Although vaccine coverage rates varied, herd protection was observed, accounting for a fraction of the observed efficacy.[159] In a subsequent trial among Peruvian military recruits, short-term efficacy in protecting against E1 Tor cholera in the first 2 months after vaccination was 86%.[160] A recent study assessing the effectiveness of this vaccine among a population with a high prevalence of HIV/AIDS in Mozambique revealed similar results.[161] This vaccine was found to offer 78% short-term protection against more severe cholera. Such short-term protection rates may be helpful in preventing outbreaks of cholera. The duration of this protection is currently under investigation.[162]

The efficacy of the CVD 103-HgR vaccine was measured in volunteer challenge studies conducted in North America. Efficacy is 62–64% against any diarrhea when challenged with E1 Tor strains and 100% when challenged with classical strains.[154] Duration of protection has been documented out to 6 months, but has not been tested beyond that. Curiously, the antibody response to CVD 103-HgR is higher in persons with blood group O, though it is unclear whether they are better protected.[155] A small trial in Mali found that a single dose of this vaccine was safe and immunogenic in both HIV and non-HIV infected individuals.[161] This single-dose immunization, given its relative high level of early protection, has been a candidate for use during an outbreak, however is not currently available on the market due to discontinued production.

The vibriocidal response of the bivalent, anti-O1, anti-O139 whole-cell vaccine was evaluated in Vietnam. This two-dose regimen was directly compared with the WC/rBS

Table 4. Characteristics of Cholera Vaccines Now in Commercial Production

Characteristic	Type	Route	Number of doses	Time to complete primary series	Efficacy	Duration
Whole cell/B-subunit	Killed organisms/ B-subunit of toxin	Oral	3[a]	12 weeks[a]	62% at 1 year[a,b]	2 years
Whole cell	Killed organisms	Oral	2			

[a]In Bangladesh field trials. Recent field trial in Peru used two doses at 1- to 2-week intervals, and obtained efficacy at 1–2 months of 86%.[154]
[b]Against both classical and E1 Tor strains. Protection against E1 Tor alone was reported to be lower.

in both an adult and pediatric population. A rise of at least fourfold anti-O1 vibriocidal response was noted in nearly 60% of recipients, similar to the response elicited by WC/rBS. The serum anti-O139 response was smaller, however, both responses were greater in children than adults.[153]

Historically, cholera vaccine has not been used successfully as a control measure in the epidemic setting. The risk of cholera is immediate, whereas protection afforded by vaccines begins a week or two after completion of the primary series. In an extreme example, the cholera epidemic that occurred among refugees at Goma, Zaire, was largely over in 4 weeks, before a two-dose WC-BS vaccine could have provided protection, even had a vaccine campaign commenced the day the camp was created. The single-dose administration of CVD 103-HgR makes it a more feasible option. Indeed, during a cholera outbreak in Pohnpei Island, Micronesia, in 2000, the government attempted to use a mass immunization strategy to limit the spread of disease. Nearly 50% of the population was vaccinated with a retrospective calculated vaccine efficacy of 79%.[163] The rate of suspect cholera was five times lower among those who received the vaccine when compared to their unvaccinated counterparts. Although traditional hygiene control measures continue to be the most effective method of limiting the spread of cholera, the evidence in Micronesia suggests that mass vaccination may have a complementary role.

The availability of vaccines leads to pressure to use them, and the issue illustrates what to expect when partially effective vaccines become available for other feared diseases such as acquired immunodeficiency syndrome (AIDS). Humanitarian impulses, coupled with the need for public health departments to "do something," encourage cholera vaccination campaigns. However, experience has shown that once a campaign is launched, pressure to vaccinate everyone can be irresistible, and scarce public health resources are diverted from other critical activities. It will be difficult to convey the notion of partial protection to a population accustomed to thinking of vaccines as "magic bullets"; vaccines may falsely believe themselves to be completely protected and may ignore other prevention advice. Vaccination is not the only means of preventing cholera, and needs to be shown to be less expensive and more effective than other strategies for preventing cholera before it can fulfill its full potential as a public health tool.

10. Unresolved Problems

Fundamental questions about the nature of the environmental reservoir remain unanswered. We do not know the nature of these niches, nor how *V. cholerae* persists from one season to the next, nor what determines the sharp seasonal increases in disease. The failure of the Latin American epidemic to even appear in the Caribbean suggests there could be an ecological resistance to introduction. The role of bacteriophage in bringing epidemics to an end, debated since the 1930s, remains to be defined and exploited.[74] Better ecological understanding may lead to improved prevention strategies.

Major questions remain about the bacterial pathogen itself. The mysterious advance and decline of the classical biotype of *V. cholerae* O1 and the potential for competitive interactions among the various strains are of great potential importance. Will *V. cholerae* O139 Bengal persist and continue to spread, becoming the eighth pandemic? The microbial characteristics that permit epidemic spread are undefined. The appearance of *V. cholerae* O139 shows that immunologically important changes can occur in the O-antigen without altering epidemic potential. The appearance of *V. cholerae* O139 offers a case study in the evolution of bacterial serotypes in general. This may be a bacterial example of "antigenic escape" mutation, like the changes that occur regularly in influenza viruses, an evolutionary response by a pathogen to high levels of immunity. Does this change allow this organism to spread more rapidly in a population that was previously immune to cholera because of repeated exposure to O1 strains? Will it continue to spread in less immune populations, where it may have less of an advantage over O1 strains? Will *V. cholerae* O141 cause an epidemic?

Many questions remain unresolved about cholera in relation to the human environment. The development of specific and sustainable control measures is a continuing challenge. Appropriate technologies for water treatment and storage, for sanitation, and for safer food handling are likely to have a direct impact on cholera as well as on a variety of other diseases transmitted through contaminated water and food. In 1854, John Snow demonstrated that water pipes were dangerously efficient at delivering cholera throughout London. Now, throughout the developing world, poorly maintained urban water systems are just as dangerous. Rebuilding such systems depends on the confidence and participation of people in their local government, as well as on the funds for construction and repair. The development of sustainable and locally controlled means of water treatment, such as the combination of better water storage vessels and water disinfection by the consumer at the point of use, may be an interim solution that can help communities gain experience in providing necessary services for themselves.

The search for better cholera vaccines is accelerating. We are soon likely to have a substantially improved understanding of virulence mechanisms and of the nature of the protective immune response. Vaccine research is hampered

by the lack of an animal host (so that volunteer trials are inevitable) and is now complicated by the appearance of *V. cholerae* O139. Vaccines may now need to be polyvalent, providing protection against both O1 and O139 serogroups. However, continued progress means that promising vaccines are available, with others on the horizon, and the goal of sustained protection may be achievable.

Epidemic cholera is an indicator of severe underdevelopment, a sign that the basic sanitary infrastructure of a society is below a critical threshold that guarantees its citizens safe water and sewage disposal. In late 2008, Zimbabwe experienced one of the largest cholera outbreaks reported to date. As of February 16, 2009, over 75,000 cases of cholera and 3,600 deaths have been recorded. A host of factors, including a deteriorating health infrastructure, decreased awareness regarding the prevention and treatment of cholera, and a delayed outbreak response, are to blame.[164,165] Communities that make the necessary investments will prevent this and many other diseases that are transmitted through the same routes. Bringing about this investment worldwide, a sustained global "Sanitary Revolution," is a central challenge for global public health in the 21st century. The scope of cholera prevention extends beyond public health and clinical medicine to include fisheries, agriculture, shipping, and tourist industries of many nations. Just as cholera spurred the development of sanitary reform and modern public health in Europe and America, so epidemic cholera is likely to be less and less tolerable in the growing democracies of the developing world.

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CHAPTER 13

Clostridium difficile

Dale N. Gerding and Stuart Johnson

1. Introduction

Clostridium difficile produces gastrointestinal infection in humans and animals, ranging from asymptomatic colonization to diarrhea, pseudomembranous colitis, toxic megacolon, colonic perforation, sepsis, and death. *C. difficile* has emerged as a major cause of nosocomial diarrhea and is the most frequently identified cause of hospital-acquired infectious diarrhea in patients.[1,2] *C. difficile*-associated disease (CDAD) is a general term used to describe the illness caused by this organism, which is usually diarrhea, but also includes abdominal pain, fever, and absence of diarrhea. Pseudomembranous colitis (PMC) is a descriptive term for the inflammatory colitis with grossly visible colonic exudate composed largely of proteinaceous material and cellular debris. *C. difficile* is the most commonly identified cause of PMC. Not all patients with CDAD have PMC, but PMC is considered a more severe form of diarrheal illness caused by *C. difficile*. This species has been unprecedented in the bacteriologic world for producing disease almost exclusively in animals or humans who have been exposed to antimicrobial or antineoplastic agents; however, recent reports suggest that some cases may not be associated with antimicrobials and that anti-acid medications such as proton pump inhibitors and H_2 receptor antagonists may predispose to CDAD.[3,4] The relative importance of these agents as risk factors for CDAD and the role of gastric acidity in the host defense against this spore-forming pathogen is not clear.

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2. Historical Background

The historical evolution of the discovery of *Bacillus difficilis* by Hall and O'Toole[5] in 1935 in the feces of asymptomatic newborn infants to the eventual linking of the organism to the new disease, "clindamycin colitis," in the 1970s is a fascinating story of the marriage of an organism without a disease to a disease without an organism. As outlined by Bartlett,[6] three diverse investigative areas, beginning with the first description of PMC by Finney[7] in 1893, the discovery of *C. difficile* by Hall and O'Toole[5], and the description of the rodent model of antibiotic-associated colitis by Hambre et al.[8] in 1943, were critical to the ultimate discovery of *C. difficile* as the major cause of PMC.

Pseudomembranous colitis was recognized historically before antibiotics were discovered and was noted as a rare complication of gastrointestinal surgery. The increasing use of antibiotics was accompanied by an increase of PMC cases. Chloramphenicol and tetracycline were the most frequently implicated agents, but "staphylococcal enterocolitis" was the usual diagnosis based on the Gram's stain evidence of typical gram-positive cocci in the stool. It has not been established whether *C. difficile* was the cause of at least some of this illness, but the subsequent publication of the use of oral vancomycin to successfully treat this disease is provocatively suggestive of the possibility.[9] Pathologically, staphylococcal enterocolitis involved both large and small bowels, whereas *C. difficile*-associated PMC is confined to the colon, so they may be separate diseases as confirmed by documentation of both distinct diseases simultaneously in the same patient.[10] In 1974, Tedesco et al.,[11] using endoscopy, reported an extraordinary high rate of PMC in patients who had received clindamycin, leading to an intensification of the search for the etiology.

The discovery that antibiotics produced lethal illness in guinea pigs and hamsters was first observed by Hambre

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et al.[8] using penicillin to treat gas gangrene in guinea pigs. At autopsy these animals demonstrated a consistent finding of a dilated inflamed cecum filled with liquid stool. Although the cause of the illness was vigorously pursued, no etiology was discovered, and the connection of this animal model to human antibiotic-associated enterocolitis was not made. Cytopathic toxicity for cell lines used for viral culture of animal stool extracts and later human stools from patients with PMC led to the mistaken postulate of a viral etiology.[12] Protection of animals challenged with clindamycin by giving them oral vancomycin implicated gram-positive bacteria as causative and led to the postulate of a toxin etiology for the cell cytotoxicity.[13,14] Eventually a clostridial toxin-producing organism was implicated as the cause of both hamster and human diseases.[15,16] Subsequent work disclosed that two large toxins, A and B, are responsible for causing clinical illness and that the majority of clinical illness in humans occurs in hospitalized patients, particularly the elderly.[17]

3. Methodology

3.1. Sources of Mortality Data

Mortality data are based on surveys and reports from multiple hospitals and ranged from 0.6 to 1.5% of patients who acquired CDAD prior to 2000.[18–19] Directly attributable mortality reported with an epidemic strain of *C. difficile* was 6.9% during a multiple hospital outbreak in 2004 in Quebec, Canada.[20]

3.2. Sources of Morbidity Data

CDAD and PMC were not reportable illnesses previously, but are now reportable in Quebec and the United Kingdom. Rates of CDAD are also reported anecdotally in the medical literature primarily from institutional experiences and range from 0.3 to >22.5 cases per 1,000 hospital discharges.[20–25] CDAD rates include cases of PMC, but the proportion of CDAD patients who have PMC is not clear. In one well-studied group of 67 patients with CDAD (diarrhea plus stool culture and toxin assay positive) who underwent flexible sigmoidoscopic examination, PMC was found in 51%.[17] Major morbidity includes colectomy for uncontrolled disease which occurred in 1.9% of patients in the 2004 Quebec outbreak.[20]

3.3. Surveys

Population survey data for CDAD are very limited and serological data are not useful. *C. difficile* stool culture surveys are reported sporadically. Most surveys reveal that less than 5% of stools of asymptomatic adults contain the organism (range 1–15%)[26,27]; however, it is commonly present asymptomatically in infants under 1 year of age (range 15–70%).[8–30]

3.4. Laboratory Diagnosis

3.4.1. Toxin Detection. Detection of cell cytotoxicity in diarrheal stool that is neutralized by the addition of specific *C. difficile* antitoxin prepared from sera of immunized animals (or *C. sordelli* antitoxin, which cross-reacts with *C. difficile* antitoxin) is the most specific diagnostic test for CDAD. Most cell lines used for viral isolation are suitable for detection of the *C. difficile* cytotoxin, toxin B.[31] Proper dilution of the specimen is required for optimal results (dilutions usually range from 1:40 to 1:200); lower dilutions can result in nonspecific cell toxicity and false-positive results. Higher dilutions may result in false negatives.[31] Sensitivity of the cell cytotoxin test ranges from 67 to 100%, somewhat lower than the sensitivity of organism culture.

Commercial enzyme immunoassays (EIA) for the detection of toxin A and/or toxin B are also available and have undergone evaluation and publication in peer-reviewed journals. The EIA test kits employ monoclonal and/or polyclonal antibodies against the toxin(s) for detection. These tests are widely used in US hospitals for the detection of toxin but are less sensitive than cell cytotoxicity and organism culture; however, they can be performed more rapidly.[31] The first generation of EIA tests detected only toxin A and were not able to detect some toxin variant strains. Many clinical laboratories have since switched to EIA tests that detect both toxins A and B; however, these newer assays still may only be 80% sensitive.[32]

3.4.2. Organism Detection. Stool culture for *C. difficile* is the most sensitive laboratory test available, but it is not as specific as cell cytotoxin testing, primarily because there are often many patients in hospitals who are asymptomatically colonized by toxigenic or nontoxigenic strains of *C. difficile*. Culture of *C. difficile* from such a colonized patient who has diarrhea from another cause could be misinterpreted as CDAD. For this reason, confirmation of the ability of an organism to produce toxin in vitro has been suggested as a means to improve the specificity of culture as a diagnostic test in patients who have negative stool toxins or have not had a stool toxin test performed.[31,33] Selective agar medium containing cycloserine, cefoxitin, and fructose (CCFA) is required for the recovery of *C. difficile* from stool specimens; preplacement of CCFA plates in an anaerobic atmosphere for at least 4 hours prior to specimen inoculation enhances organism recovery.[34,35] Stool culture sensitivity is 89–100% and specificity is 84–99%.[31] Stool culture

for *C. difficile* is essential for epidemiological investigation of outbreaks so that organism typing can be performed.

Polymerase chain reaction (PCR) employing arbitrary primers is a sensitive method of organism detection; if the primers are selected from within the toxin gene sequences it is specific for detection of toxigenic *C. difficile*.[36–39] Real time (RT) PCR has recently become available as a diagnostic test and looks very promising as a sensitive, specific and rapid test, but is still undergoing clinical comparative analysis for sensitivity and specificity.

The latex agglutination test for detection of *C. difficile* protein was originally developed to detect toxin A, but subsequently was found to detect another protein, glutamate dehydrogenase.[40,41] Although rapid, inexpensive, and reasonably specific, the sensitivity of this test (58–68%) was too low, but has been improved by the use of an enzyme immunoassay method for detection that has improved sensitivity.[31]

3.4.3. Detection of Pseudomembranous Colitis. Direct visualization by sigmoidoscopy or colonoscopy is the usual method used to diagnose PMC. Small lesions may require biopsy and histological examination for diagnosis. Visualization of PMC is observed in only 51–55% of patients who have diarrhea and have *C. difficile* and its toxin detected in the stool; hence, although endoscopy is essential for the diagnosis of PMC, it is not sufficient to diagnose all cases of CDAD.[17,42]

3.4.4. Pathology. Grossly, PMC appears as a studding of the colonic mucosal surface with white to yellow adherent raised plaques. The distribution of lesions usually includes the whole colon, but it may be variable with occasional rectal sparing. Lesions end abruptly at the ileocecal valve. Microscopically, the membrane is composed of fibrin, mucus, leukocytes, and cellular debris and demonstrates a point of attachment to the mucosal surface. There is a predominantly neutrophilic inflammation of the underlying mucosa, which is typically quite focal and patchy. The superficial epithelium is quite commonly eroded, with necrosis present in the upper half.[43]

4. Biological Characteristics of the Organism (Etiologic Patterns)

C. difficile is a gram-positive anaerobic spore-forming bacillus. Spores are resistant to drying and persist in the environment for prolonged periods of time. The organism is widespread in distribution worldwide and is found in soil, hay, mud, sand, and the stools of multiple animal species including horses, dogs, cats, camels, donkeys, and cattle. The ability of the organism to produce disease is attributed to two large exotoxins: toxin A, with a molecular weight of 308 kDa, and toxin B, with a molecular weight of 207 kDa.[44] Toxin A is a large single-unit toxin that possesses both enterotoxic and cytotoxic properties. Toxin A binds to carbohydrate receptors on the cell surface and mediates toxicity by disruption of the cell cytoskeleton through glucosylation of small GTP-binding proteins of the Rho subfamily. Toxin A is also a potent chemoattractant for granulocytes. The toxin B receptor is unknown, but the toxin is a very potent cytotoxin with a similar or nearly identical mechanism of action to that of toxin A. Toxins A and B exhibit significant homology (44.8% identity) with the major differences at the C-terminus region of putative receptor binding. Most *C. difficile* organisms either possess the genes to produce both toxin A and toxin B or lack both genes. A variety of toxin variant strains of *C. difficile* have been identified and have been documented to cause human CDAD and a toxinotyping system has been developed to describe these variants.[45] Toxinotype VIII strains produce no toxin A, but produce a modified toxin B (A-B+ strains) and are capable of causing the full spectrum of CDAD disease, including fatal PMC.[46] The prevalence of toxinotype VIII strains in most hospitals is probably low,[47] but they have caused CDAD hospital epidemics worldwide and are not detected in laboratories that only use toxin A EIA tests for diagnosis.[48,49]

A major CDAD epidemic in the United States, Canada, and Europe has been caused by a toxinotype III variant strain of *C. difficile* that also produces a binary toxin (actin-specific ADP-ribosyltransferase) in addition to toxins A and B and has a deletion in the *tcdC* gene, a putative negative regulator of toxin A and toxin B production.[20,50] These strains have been associated with increased mortality and morbidity and produce about 20 times as much toxins A and B in vitro as standard *C. difficile* strains.[51] Strains that lack the ability to produce toxins entirely are not considered to be pathogenic and have not been associated with clinical illness.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

There are relatively few studies of the incidence of CDAD in the community setting. Most studies have been conducted in hospitals or chronic care facilities. One community-based retrospective cohort study of members of a health maintenance organization identified a CDAD incidence of 7.7 per 100,000 person-years.[52] The rate of disease resulting in hospitalization was 0.5–1.0 per 100,000

person-years and is comparable to the rate of 1.4 per 100,000 person-years published earlier.[53] Two studies from Perth, Australia, have determined the proportion of community-acquired diarrhea that is caused by *C. difficile* using stool specimens submitted to a central laboratory by general practitioners in the community as a denominator.[54,55] The incidence of *C. difficile* was 5.5% in the first study, 2.6% in the first phase of the second study, and 10.7% in the second phase following an educational program for the referring physicians.[54,55] *C. difficile* was the most common or second-most common pathogen isolated in the two studies. Anecdotal observations in the United States suggest more severe CDAD in previously low-risk populations (peripartum women and community residents), and a primary care database study in the United Kingdom suggests that community-associated cases of CDAD are increasing.[4,56] Both these observations require validation.

The incidence of CDAD in hospitals is considerably higher than in the community and has been studied in two large teaching hospitals over a 10-year period. At the Minneapolis Veterans Affairs Medical Center, the annual CDAD incidence between 1982 and 1991 varied from 3.2 to 9.9 cases per 1,000 patient discharges (0.30–0.71 per 1,000 patient days).[18,25] A remarkably similar incidence was found at the Sir Charles Gairdner Hospital in Perth from 1983 to 1992, where the annual CDAD rate ranged from 0.22 to 0.55 per 1,000 patient days.[57] Both hospitals had active laboratory detection programs utilizing both culture and cytotoxin testing, but the US hospital had a more rigorous CDAD clinical correlation with patient symptoms. Virtually all patients were adult males in the veterans hospital population. A 1997 survey of 18 Canadian hospitals found an incidence of CDAD of 6 cases per 1,000 discharges, contrasting sharply with the rate of 22.5 per 1,000 discharges in 12 Quebec hospitals during an outbreak with the epidemic strain in 2004.[19,20]

Prevalence data are available for the presence of *C. difficile* in stool specimens of persons without diarrhea. For healthy adults, the prevalence rate of asymptomatic*C. difficile* carriage has been found to be under 3%.[26,30] Prevalence of *C. difficile* in a home for the aged (mean age 86 years) on three surveys was 2.3, 2.1, and 8.1%, respectively, whereas in an adjoining chronic care hospital it was 14.7, 7.1, and 10.2%, rates comparable to other reports of residents of long-term care facilities.[58] A point prevalence rate of 10.8% was found in a survey of 340 asymptomatic adult patients in the acute care Minneapolis Veterans Affairs Medical Center (D. Gerding, unpublished). The prevalence of *C. difficile* in the stool of infants under 1 year of age ranges from 15 to 70%, decreasing to adult levels by the age of 3 years.[28–30]

5.2. Epidemic Behavior and Contagiousness

Epidemics have not been described outside of institutional settings. In general, only individuals who have received antimicrobial or antineoplastic agents are susceptible to CDAD. Reports of CDAD in association with proton pump inhibitors or in the absence of antimicrobials are of concern and should be confirmed with more studies.[4,56] Epidemics within acute and chronic care institutions are well described and have been attributed to a single predominant strain of *C. difficile* as well as to several strains present simultaneously.[20–22,59–61] Outbreaks are characterized by culture of the organism and typing by a variety of methods, which include DNA restriction endonuclease analysis (REA), pulsed field gel electrophoresis, arbitrary-primed PCR, PCR ribotyping, and immunoblot methods.[20–22,37–39,50,51,59,60] Contagiousness has been difficult to assess because of the variable susceptibility of exposed persons, the widespread environmental distribution of the spores in epidemic settings, and the possible variable virulence characteristics of the organism. Animal studies indicate that the spore inoculum required to produce disease is <100 colony-forming units (CFU) in susceptible hosts who have received antimicrobials.

5.3. Geographic Distribution

C. difficile and CDAD are considered to be worldwide in distribution, but documentation of rates in many Asian and African countries has not been established.

5.4. Temporal Distribution

No seasonal variation in the incidence of CDAD has been identified, although hospital case rates frequently rise in the winter respiratory infection season in the Northern Hemisphere, possibly related to increased antimicrobial use.

5.5. Age

The presence of *C. difficile* in stools is highest for children under 1 year of age, but these children are nearly all asymptomatic.[28–30] The rate of CDAD has been found to increase with age, being highest in the eighth and ninth decades.[20,26,57] The rate of cytotoxin found in stools was also shown to be highest in the ninth decade.[26] McFarland et al.[62] identified increased age as a risk factor for CDAD, with a relative risk of 11.77 (2.46–56.39, 95% CI) from ages 61–73 years and with a relative risk of 14.30 (3.27–62.51, 95% CI) over the age of 75 years. This was confirmed by Loo et al.[20] who found the CDAD rate to be 74.4 per 1,000 discharges for hospitalized patients in their nineties.

5.6. Gender

Women were found to have significantly higher rates of CDAD between ages 21 and 50 years and over the age of 70 years in one study.[26] In another large hospital study, the ratio of females to males with CDAD was 1.6:1 for ages 40–59 years and 1.7–2.2:1 over the age of 70 years,[57] despite ratios of female to male patients of 0.9:1 in all but the highest decade. Other studies have shown no difference in CDAD rates between the sexes.[23,62] A large case–control study of 237 patients in Quebec hospitals had 48.5% male patients which was not statistically different from the control group.[20]

5.7. Race

No race-specific attack rate data for CDAD are available. Fecal isolation rates for a Japanese population were higher (15%) than in Sweden (1.9%).[26,27]

5.8. Occupation

No occupational data regarding risk are available. Health care workers have not been noted to be at increased risk of CDAD, although sporadic cases in health care employees do occur, almost always in association with antimicrobial use. Health care workers do not have an increased rate of asymptomatic *C. difficile* colonization.[17,63]

6. Mechanisms and Routes of Transmission

The exact route of transmission is not known, but is assumed to be by way of oral ingestion of *C. difficile* spores, which are found in water, soil, and animal feces. Nosocomial transmission accounts for the majority of CDAD cases reported in the literature. Two potential reservoirs for spread to patients are known: infected patients (symptomatic and asymptomatic) and a contaminated environment including inanimate objects. Infected patients, the environment, and health care workers are intertwined in the nosocomial transmission process, with hand contamination of health care workers correlated with environmental spore contamination, but the exact mechanism(s) by which transmission occurs still remains obscure.[64] It is well documented that patients acquire *C. difficile* in the hospital after being admitted free of the organism.[21,22,65] The rate of acquisition over time has been shown to be linear at about 8% per week on one ward of a hospital with an annual CDAD rate that varied from 3.2 to 9.9 cases per 1,000 discharges.[65] Most of these acquisitions occur asymptomatically.[21,22,65] Asymptomatic stool carriage of *C. difficile* in hospitals with this rate of CDAD has been found to be approximately 20% of all admitted patients.[17,21,22,65] By using genetic typing techniques, it has been shown that the frequency of CDAD among patients who acquire *C. difficile* in hospital is dependent on the strain acquired, but that even highly virulent strains are asymptomatic in the majority of patients.[22] Admissions of *C. difficile*-colonized patients to a hospital ward were associated temporally with the subsequent acquisition of the identical strain by other patients already in the ward.[65]

The hospital environment is contaminated with *C. difficile* spores in proportion to the status of the patient in the environment: lowest around patients who are free of *C. difficile*, intermediate in rooms of patients asymptomatically colonized, and highest in rooms of patients who have CDAD.[21,66] Persistence in the environment has been documented for 5 months.[66] Inanimate objects in the environment have been implicated as the source of transmission, including contaminated commodes, bathing tubs, and rectal thermometer handles.[67–69]

The hands of hospital personnel have been found to be contaminated with *C. difficile*, and several lines of evidence suggest that personnel contamination of the hands may be an important means of transmission.[21,66] In one outbreak, six of seven new cases of CDAD caused by a specific *C. difficile* strain occurred in patients cared for by the same surgical team caring for a long-term colonized patient with a colostomy.[22] A controlled trial of the use of vinyl gloves by health care personnel handling body substances showed a significant decrease in new CDAD cases, suggesting hands as a means of transmission.[70] It is not clear how personnel' hands become contaminated, as both the patients and their environment could be the source.

7. Pathogenesis and Immunity

We postulate that at least three factors are required for CDAD to occur: (1) antimicrobial exposure, which presumably disrupts the normal protective colonic flora; (2) acquisition of a toxigenic *C. difficile* organism; and (3) host factors, specifically the ability to mount a serum IgG antibody response against toxin A that determines if CDAD or asymptomatic colonization will occur (Figure 1).[71,72] Two major virulence factors have been identified in *C. difficile* organisms—toxin A and toxin B—and virtually all organisms either possess both toxins or neither. The

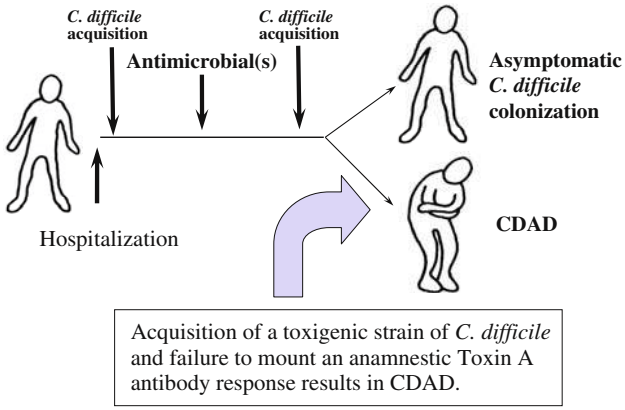

C. difficile acquisition
C. difficile acquisition
Antimicrobial(s)
Asymptomatic C. difficile colonization
CDAD
Hospitalization
Acquisition of a toxigenic strain of C. difficile and failure to mount an anamnestic Toxin A antibody response results in CDAD.

Figure 1. Schematic of the hypothesis for the pathogenesis of *Clostridium difficile*-associated disease (CDAD).[71,72] Patients acquire *C. difficile* spores from contact with health care workers or the environment, but will not become ill with CDAD unless they acquire a toxigenic strain, and are exposed to antimicrobials that disrupt the normal protective flora and fail to raise an anamnestic serum IgG antibody response to toxin A. Patients who quickly develop a high antibody response will become colonized but remain asymptomatic.

importance of a third toxin, binary toxin, found in some strains including the recent epidemic toxinotype III strain, is unknown. Toxin A-negative and B-negative *C. difficile* organisms possessing binary toxin do not cause disease in hamsters, but culture supernatants produce a positive assay in rabbit ileal loops.[73,74] Nontoxigenic organisms are not considered capable of producing CDAD, but toxigenic strains appear to differ in their ability to produce CDAD, possibly on the basis of ability to produce toxins; however, in vitro production of toxin has correlated poorly with disease in the hamster model.[22,51,75,76]

Evidence for host factors in the pathogenesis of CDAD include the observation that asymptomatic colonization, including colonization with toxigenic and epidemic strains, is common and appears to be associated with significant protection against subsequent CDAD.[77] The immune response is a potentially important host factor and some indirect evidence supporting a role for immunity has been found in the pediatric population. Among children with chronic diarrhea, *C. difficile* is isolated more often among those with hypogammaglobulinemia than among those with normal immunoglobulin levels.[78] Moreover, children with chronic relapsing *C. difficile* colitis who had low levels of antitoxin A IgG showed both clinical and microbiological responses and increased levels of antitoxin A IgG following treatment with intravenous gamma globulin.[79] Patients with CDAD develop systemic and mucosal antibody responses to toxin A.[80,81] Failure of patients to raise their serum IgG antibody response promptly to toxin A when infected with a toxigenic strain of *C. difficile* has correlated with CDAD symptoms and with recurrence of CDAD.[71,82] In contrast, patients who raise their serum IgG antibody response to toxin A remain asymptomatic following acquisition of the organism, and those who raise their serum IgG antibody response to toxin A during treatment are less likely to have a recurrence.[71,82] Some investigators have found lower antitoxin A levels in patients with prolonged diarrhea and in those with relapsing CDAD than in patients with diarrhea of shorter duration and in those with CDAD limited to one episode.[83]

8. Patterns of Host Response

8.1. Clinical Features

Most patients with symptomatic *C. difficile* infection present with diarrhea. Fever is present in 28%, abdominal pain in 22%, and leukocytosis (>10,000/mm^3) in 50% of the patients.[17] A marked leukocytosis which frequently exceeds 20,000/mm^3 may indicate a bad prognosis, particularly if it continues to rise during treatment.[84] Very rarely, patients present with no prior diarrhea and have abdominal pain and tenderness, decreased bowel sounds, and an ileus pattern on abdominal X rays. Abdominal CT scans frequently reveal markedly thickened colonic mucosa and ascites in patients with ileus and severe or fulminant CDAD. Severe complications include toxic megacolon and colonic perforation, both of which are rare but life threatening. Rare cases of extraintestinal infection with *C. difficile* have been reported and include splenic abscess, bacteremia, osteomyelitis, cellulitis, and reactive arthritis.[85,86]

8.2. Diagnosis

The diagnostic definition of CDAD should include (1) diarrhea (variously defined as six watery stools in 36 hours, three or more unformed stools in 24 hours for 2 days, or eight unformed stools in 48 hours); (2) PMC visualized by endoscopy, or toxin A or B detected in stool, or stool culture positive for toxin-producing *C. difficile*; and (3) no other recognized etiology for diarrhea. In addition, a history of antimicrobial administration within the previous 8 weeks is present in nearly all patients, but is not included in the diagnostic definition to allow comparison of antimicrobial use as a risk factor. Endoscopy is the only means to diagnose PMC, but the sensitivity of endoscopy in patients with CDAD is only

51–55%, and the procedure is costly and invasive.[17,42] Most diagnoses of CDAD are made by stool laboratory testing; the cell cytotoxin test is most specific, but culture is the most sensitive. Most clinical laboratories in the United States test for toxin A or toxins A and B using an enzyme immunoassay method which is not as sensitive as the cell cytotoxin test or stool culture for *C. difficile*.

9. Control and Prevention

9.1. General Concepts

Efforts to control and prevent CDAD in institutions have been frustratingly difficult and remain a major area of needed research. Control measures have been of two types: those designed to prevent the patient from acquiring the organism and those designed to minimize risk of clinical illness if the organism is acquired. Barrier precautions are recommended to prevent transmission and include use of gloves by personnel, which has been shown to be effective in one hospital trial,[70] and isolation, cohorting, and hand washing, which are recommended but have not undergone rigorous clinical trials of efficacy. Alcohol hand rubs are ineffective in removing spores of *C. difficile,* therefore hand washing with soap is the preferred hand hygiene method. Efforts to reduce environmental contamination by disinfection and cleaning with sporicidal agents such as hypochlorite solution (1:10 diluted bleach) have demonstrated efficacy in reducing CDAD rates on a bone marrow transplant unit, reducing the CDAD rate from 8.6 to 3.3 cases per 1,000 discharges in one clinical trial.[87] Identification and removal of contaminated inanimate objects such as electronic rectal thermometers have been demonstrated to effectively reduce transmission.[69] Treatment of asymptomatic patients with metronidazole or vancomycin has been proposed as a means to prevent these patients from being a source of contamination of the environment and other patients, but metronidazole has been shown to be ineffective and vancomycin to be only transiently effective in reducing *C. difficile* in the stool.[88] Among the methods directed at reducing the risk of CDAD, the most effective has been control of antimicrobial usage, particularly clindamycin, which has been particularly effective in controlling outbreaks caused by clindamycin-resistant *C. difficile*.[59,89] Control of second and third generation cephalosporin use has also been associated with reduced CDAD rates.[90,91] Outbreak strains since 2000 in the United States, Canada, and Europe are resistant to newer fluoroquinolones, moxifloxacin, and gatifloxacin, and receipt of a fluoroquinolone antibiotic has been a risk factor for CDAD in hospitals experiencing outbreaks with this strain.[20] In one long-term care institution harboring the epidemic strain, CDAD rates decreased markedly when levofloxacin was substituted for gatifloxacin.[92]

9.2. Prophylaxis

Prophylactic treatment of patients receiving antimicrobials in order to prevent CDAD has been attempted in a clinical trial using a yeast, *Saccharomyces boulardii*, which significantly reduced antimicrobial-associated diarrhea, but did not reach statistical significance in reducing CDAD.[93] A review of the use of probiotics for prevention and treatment of CDAD concluded that there is insufficient evidence for the routine clinical use of probiotics to prevent or treat CDAD.[94]

9.3. Immunizations

No immunization against CDAD is currently available, but a vaccine is under development.

10. Unresolved Problems

10.1. Epidemiology

The major epidemiological question that is unresolved is the mechanism of *C. difficile* transmission in health care institutions. In addition, there is a marked need for simple, rapid, and inexpensive diagnostic tests that will increase the rapidity of patient diagnosis and thus reduce infection transmission. Much of traditional epidemiology is focused on clinical disease recognition and isolation and cohorting of the clinically ill. It is unclear what role asymptomatic patients play in the transmission process. If it were easier to identify these patients, their role in disease transmission could be better defined. Rapid, inexpensive, and sensitive organism typing methods are needed as a tool to better define the molecular epidemiology and transmission of *C. difficile.*

10.2. Control and Prevention

Traditional barrier methods other than wearing gloves have not been particularly effective in controlling *C. difficile* in hospitals. Efforts to more appropriately utilize antimicrobials will help to reduce undue risk to patients, but antimicrobial use in today's high-technology intensive care hospital environment is unavoidable and essential for treatment of some patients. Vaccines and colonization by nontoxigenic strains of *C. difficile* hold promise.[77] Efforts to better define the role of immunity could result in innovative vaccine

development. Prophylactic measures to prevent disruption or reconstitute the bowel flora should also be considered.

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CHAPTER 14

Diphtheria

Melinda Wharton

1. Introduction

Diphtheria is an acute infectious and communicable disease caused by *Corynebacterium diphtheriae*. The organism infects primarily the respiratory tract, where it causes tonsillopharyngitis and/or laryngitis, classically with a pseudomembrane, and the skin, causing a variety of indolent lesions. If the infecting strain produces exotoxin, organ damage, especially myocarditis and neuritis, may ensue. The incidence of the disease has declined to low levels in most industrialized nations due to mass immunization with diphtheria toxoid. However, in the 1990s a diphtheria epidemic occurred throughout the former Soviet Union, resulting in more than 157,000 reported cases and 5,000 deaths. Thus, it is not safe to assume that diphtheria will remain a disease of the past.

2. Historical Background

Although possibly recognized as a clinical entity by Hippocrates (4th century BC), Aretaeus of Cappadocia (2nd century AD) gave the first clear description of respiratory diphtheria, which he called Egyptian or Syrian ulcers. Aëtius of Ameda (6th century AD) gave the first description of epidemic diphtheria and mentioned palatal paralysis as a sequel. Repeated epidemics of diphtheria (which was called *garrotillo* in Spain) were reported in Western Europe from the 15th century and in the New England states of the United States from the 16th century. Samuel Bard of New York (1771) described laryngeal diphtheria in a work entitled *Angina Suffocativa*. Bretonneau (1826) gave the disease its present name, using the Greek word for tanned skin or leather, after the tough pseudomembrane, and differentiated it from other diseases of the upper respiratory tract. Klebs (1883) identified the diphtheria bacillus by microscopic examination of diphtheritic membranes and associated it with the disease, and Loeffler (1884) first cultivated the organism on artificial media, established the causal relationship of the diphtheria bacillus by producing the disease in guinea pigs, and identified asymptomatic infections (carriers) among healthy children. Loeffler observed that the organism appeared to remain localized in the membrane without systemic invasion and hypothesized that damage to cardiac and nervous tissues was due to absorption of toxins elaborated by the organism. This was confirmed by Roux and Yersin (1888), who demonstrated that sterile filtrates of diphtheria cultures would kill guinea pigs and produce lesions identical to those that occurred after injection of the organism. Von Behring (1890–1893) showed that serum obtained from animals immunized against diphtheria toxin could be used for the prevention and treatment of the disease. For this work he received the first Nobel Prize in Medicine in 1901. Theobald Smith (1907) began the development of active immunization against diphtheria by reporting that durable immunity to the disease could be induced in guinea pigs by the injection of mixtures of diphtheria toxin and antitoxin. Schick (1913) introduced the skin test for susceptibility that bears his name. Ramon (1923) inactivated diphtheria toxin using formalin and heat, producing diphtheria "toxoid," which was non-toxic but able to induce protective antibodies. Vaccination with diphtheria toxoid was gradually introduced after 1930 and became universal in industrialized countries by the late 1940s. Freeman (1951),[1] Groman (1953),[2] and others demonstrated that avirulent *C. diphtheriae* were rendered toxigenic when infected by a specific β-phage. Pappenheimer, Gill, and associates (1952–1973)

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have clarified the molecular mechanisms involved in the pathogenesis of the disease.[3] The evolution of knowledge about diphtheria has been important in the history of medicine, significantly influencing the development of bacteriology, immunology, pathology, epidemiology, and public health.[4]

3. Methodology

3.1. Sources of Morbidity and Mortality Data

In the United States, reporting of cases of respiratory diphtheria by health care providers is mandated in all states. In some states, hospitals and other health care facilities are also required to report cases. Cases are reported electronically to the National Notifiable Disease Surveillance System (NNDSS) and published in the weekly *Morbidity and Mortality Weekly Report* (MMWR) and annually in the MMWR Summary of Notifiable Diseases. Because diphtheria is now rare, cases reported to the NNDSS are verified before publication. If the diagnosis of diphtheria is suspected, diphtheria antitoxin (DAT) for treatment of the patient is obtained from the Centers for Disease Control and Prevention (CDC), which provides DAT within the United States under an Investigational New Drug protocol.[5] A CDC epidemiologist follows the case to collect supplemental data and to determine whether it meets criteria for reporting. It is thus likely that reporting of recognized diphtheria cases and deaths is nearly complete. The CDC maintains a database with supplemental data on diphtheria cases including vaccination status, complications, and outcome and publishes periodic summaries of the epidemiology of diphtheria in the United States.[6–9]

Since 1980, cutaneous diphtheria has not been a reportable disease in the United States. However, state public health laboratories are requested to send all *C. diphtheriae* isolates from any body site, whether toxigenic or nontoxigenic, to the CDC laboratory for confirmation.[10]

Worldwide, countries report annual numbers of diphtheria cases to their World Health Organization (WHO) regional office, and these data are collated by WHO headquarters in Geneva. The timeliness and completeness of such reporting are variable.

3.2. Surveys

Population immunity to diphtheria is evaluated by measuring serum levels of diphtheria antitoxin in a sample of the population. The National Health and Nutrition Examination Survey (NHANES) is a population-based survey of a representative sample of the US civilian noninstitutionalized population, conducted by the National Center for Health Statistics. Diphtheria antitoxin levels were determined by in vitro neutralization assay of approximately 18,000 sera collected during NHANES III (1988–1994). Sixty percent of Americans 6 years of age and older had fully protective levels of diphtheria antitoxin ($\geq$0.10 International Unit [IU]/mL), but only 30% of those 60 years of age and older had protective levels.[11]

Serologic studies were undertaken in many countries following the outbreak of diphtheria in the former Soviet Union. The level of population immunity observed varied by when diphtheria immunization was introduced and when high coverage was achieved, as well as by the specific immunization schedule used. For example, diphtheria gradually came under control in West Germany during the 1950s and in East Germany by the early 1960s. Analysis of serum specimens collected in Germany during the late 1990s demonstrated that a lower proportion of adults 40–49 years of age had protective levels of diphtheria antitoxin than younger age groups—who were more likely to have received childhood immunization—and older persons, who were more likely to have contracted diphtheria in childhood. In contrast, in Finland and the Netherlands, the age groups with the highest levels of susceptibility were the elderly.[12] A similar patter is seen in England and Wales, where 29% of adults 60 years of age and older had diphtheria antitoxin levels of 0.01 IU/mL or greater; however, the proportion with fully protective titers was much lower.[13] In Australia, approximately one-third of adults 50 years and older had fully protective titers of diphtheria antitoxin, and more than 50% had titers of 0.01 IU/mL or greater.[14]

No carrier surveys have been performed in the United States for many years; in the United States, laboratories do not routinely screen pharyngeal specimens for *C. diphtheriae*, although this is still recommended in some countries such as the United Kingdom.[15]

3.3. Laboratory Diagnosis

3.3.1. Isolation and Identification of the Organism. *Corynebacterium diphtheriae* is an aerobic, non-motile, unencapsulated, pleomorphic, gram-positive bacillus. The organism has four biotypes—*mitis, gravis*, *intermedius* and *belfanti*—distinguished by varying biochemical and hemolytic reactions.

There are several excellent reviews of the recommended procedures for the isolation and identification of *C. diphtheriae*.[16–20] If the diagnosis of diphtheria is suspected, the laboratory should be informed, since on

the traditional blood agar used for culture of respiratory specimens the organism may be overgrown or confused with nonpathogenic coryneform bacteria ("diphtheroids"). In respiratory diphtheria, material should be obtained for culture using a cotton- or polyester-tipped swab from both the posterior nasopharynx and the posterior oropharynx. If possible, specimens should be taken from beneath the margin of the membrane if one is present. If transport to the laboratory will be completed promptly, dry swabs are satisfactory; for periods of up to 24 hr, a general transport medium (e.g., Stuart or Amies) should be used; for longer periods, transport in silica gel sachets is recommended.[21] In stained smears, the organisms are club shaped (Greek *korynee*, club) and may form characteristic "Chinese letter" patterns. However, diagnosis on the basis of direct smears is not reliable and should instead rely on culture and confirmatory tests.

For primary isolation, swabs should be inoculated onto both blood agar and a tellurite-containing medium, which selectively inhibits the growth of other organisms. On tellurite media, *C. diphtheriae* typically forms brownish-black or grayish-black colonies within 18–48 hr, but other organisms may also produce similar-appearing colonies. Smears of suspicious colonies can be examined under Gram's or methylene blue stain to give a presumptive report. Colonies are taken to set up toxigenicity testing and also inoculated onto Pai or Loeffler's slants for biochemical testing, in which the pattern of metabolism of sugars, nitrate, and urea is used to confirm the species and determine biotype. Several commercial identification systems are available.

Historically toxigenicity testing was carried out in vivo by cutaneous inoculation of guinea pigs or rabbits,[22] but this is now rarely performed. More common is in vitro testing using a modified version of the immunodiffusion test originally developed by Elek.[23] In this test, an antitoxin-impregnated strip is laid along the surface of a culture plate containing Elek's medium; test and control cultures are streaked perpendicularly across the strip. Toxigenic organisms are identified by lines of toxin–antitoxin precipitate in the medium. Results are available within 24–48 hr in experienced laboratories. Other modifications to the Elek test have more recently been described which may allow detection of a positive result at 24 hr or less.[24] Many reference laboratories now perform a polymerase chain reaction (PCR) assay for detection of the diphtheria toxin structural gene (*tox*).[25–27] PCR has been found to be sensitive and specific for screening primary isolates, although false-positive toxigenicity tests may occur in isolates with nonfunctional *tox* genes. For this reason, it is recommended that PCR be used in conjunction with the Elek assay, in order to confirm the production of diphtheria toxin.[28] Cysteinase and pyrazinamidase are useful screening tests to distinguish *C. diphtheriae* and the other two potentially toxin-producing species, *C. ulcerans* (which may cause a diphtheria-like illness) and *C. pseudotuberculosis*, from other species of the genus *Corynebacterium*.[18]

A number of molecular techniques, including ribotyping, pulsed-field gel electrophoresis, multilocus enzyme electrophoresis, and the random amplified polymorphic DNA (RAPD) assay, have been used in recent years to describe the molecular epidemiology of diphtheria outbreaks.[29–33] Ribotyping has been most extensively applied and standard nomenclature has been developed.[34] These techniques have been used extensively to characterize isolates from the recent epidemic in the countries of the former Soviet Union[30,35–38] as well as to demonstrate long-term persistence of circulation of *C. diphtheriae* in two communities in the United States and Canada.[39,40]

3.3.2. Serological and Immunologic Diagnostic Methods. Immunity to diphtheria presumably involves both humoral and cell-mediated immunity to multiple antigens of *C. diphtheriae*.[41] In practice, however, only the level of diphtheria antitoxin (antibodies to diphtheria toxin) is measured. Based on the level of antitoxin associated with Schick test conversion and studies of levels of circulating antitoxin in patients at the time of hospitalization with clinical diphtheria,[42–44] it is generally agreed that persons with levels below 0.01 IU/ml have no protection against diphtheria and increasing levels above that correspond to increasing levels of protection. Some authorities differentiate 0.01 IU/ml as the minimum protective level and state that levels from 0.01 to 0.1 IU/ml confer relative or partial protection, levels of $\geq$0.1 IU/ml provide more reliable protection, and levels $\geq$1.0 IU/ml confer long-term protection.[18,45] However, no level of antitoxin provides absolute protection against clinical diphtheria.[42]

Formerly, the Schick test was widely used to determine population immunity to diphtheria.[46,47] The test is performed by subcutaneous injection of a small standardized quantity of diphtheria toxin intradermally in one arm, with a control injection of diphtheria toxoid in the opposite arm. The result is determined after 24–48 hr and again 5–7 days after inoculation. A positive erythematous skin reaction to toxin but not to toxoid indicates susceptibility. The antitoxin level at which the Schick test conversion occurs is around 0.30 IU/ml.[48]

Several assays for determination of diphtheria antitoxin level in serum have been developed. The "gold standard" tests are neutralization assays: in vivo tests performed in animals and the more convenient but equally accurate in vitro cell culture methods.[49] The enzyme-linked immunosorbent assay (ELISA) is simpler and/or less costly than the neutralization tests; while the ELISA is acceptable for population

surveys, it lacks sensitivity compared to neutralization tests for antitoxin levels below 0.1 IU/ml, and therefore is of limited usefulness for assessment of the individual patient.[50] The major role of serological tests is in periodic surveys to determine levels of population immunity to diphtheria. Individual immunity is assessed in two main settings: most commonly in modern practice, to determine antibody response to diphtheria toxoid in vaccine studies or as part of an immunologic assessment, and in the setting of suspected diphtheria, to determine the patient's antitoxin level before therapeutic antitoxin is administered. If this level is later found to be high, the diagnosis of diphtheria is less likely; a low level, while consistent with possible diphtheria, does not prove the diagnosis. Antibody response to diphtheria infection is unreliable for diagnosis, since many patients do not mount a significant antibody response, and available assays in the United States do not distinguish between human antibodies and equine antitoxin used for treatment.

4. Biological Characteristics of the Organism

Humans are apparently the only natural host and reservoir of *C. diphtheriae*. Toxigenic organisms have only been isolated from man and from certain domestic animals closely associated with man, such as horses, cows, sheep, and dogs. It is believed that this represents colonization of such animals due to human contact, rather than representing a source of infection for humans. Experimental infections can be induced in a variety of animals. *C. diphtheriae* is moderately resistant to physical factors such as drying; organisms can be cultured from the environment of patients[50] and may survive in dust for up to several weeks.[51]

The major virulence factor produced by *C. diphtheriae* is diphtheria toxin. Both toxin-producing (toxigenic) and non-toxin-producing (nontoxigenic) strains exist in nature. Expression of toxin depends on infection of the *C. diphtheriae* strain by a number of lysogenic corynephages, which carry the toxin gene (*tox*). Integration of phage DNA into the bacterium allows expression of toxin, which is facilitated by low iron conditions. Nontoxigenic strains can be converted to toxigenic ones in vitro by infection with a tox^+ phage. In addition, there have been reports of carriers simultaneously infected with toxigenic and nontoxigenic strains that appear to be identical by phage typing and DNA restriction analysis, differing only in the presence of a tox^+ β-phage.[52] This suggests that conversion of nontoxigenic *C. diphtheriae* strains to toxigenicity may occur in vivo in the human pharynx. If so, the *tox* gene could be introduced into a community following the arrival of a toxigenic strain by direct spread of that strain, but also indirectly by release of the tox^+ phage, which then may spread within the resident population of circulating nontoxigenic strains.[52]

Additional virulence factors that have been described include two cell wall components: K antigens, which appear to be important in the establishment of infection, and a glycolipid named "cord factor," which disrupts mitochondrial function.

Nontoxigenic *C. diphtheriae* may cause pharyngitis without a membrane[50,53] and a localized diphtheria-like membranous tonsillopharyngitis.[44] Although such patients do not suffer toxin-induced complications such as myocarditis and neuritis, their illness may sometimes be severe and occasional deaths are reported.[8,44] Nontoxigenic *C. diphtheriae* may cause invasive disease, including endocarditis and arthritis. Such patients usually, but not invariably, have underlying risk factors.[54–59]

Resistance of *C. diphtheriae* to penicillin has not been demonstrated,[60–64] but organisms resistant to erythromycin, clindamycin, tetracycline, and rifampin have been described.[61,65–68] There is one report of erythromycin resistance in four strains isolated from respiratory carriers after a 7-day course of erythromycin.[69] The other reported erythromycin-resistant isolates have been cutaneous isolates, with plasmid-mediated resistance and associated with previous receipt of erythromycin by the patient or with widespread use of this antibiotic for treatment of pyoderma in the community.[70] More recently intermediate resistance to erythromycin has been reported in some isolates from epidemic areas in South Asia and isolates from sporadic cases in Australia.[19] Rifampin resistance was documented in isolates from two members of a single family in Estonia.[61]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

In industrialized nations, until the 1920s–1930s, up to 10% of children in urban areas developed clinical diphtheria. Asymptomatic infection was even more common, and by 15 years of age, 70–80% of children in cities such as New York, Baltimore, and London were immune to diphtheria.[47,71,72] Diphtheria incidence in the United States (but not in England and Wales) declined before the advent of widespread diphtheria vaccination, probably because transmission was reduced as improving social conditions resulted in reduced crowding and improved hygiene. The increasing implementation of diphtheria toxoid vaccination from the late 1920s and its universal use from the late 1940s led to a steady decline in diphtheria incidence.

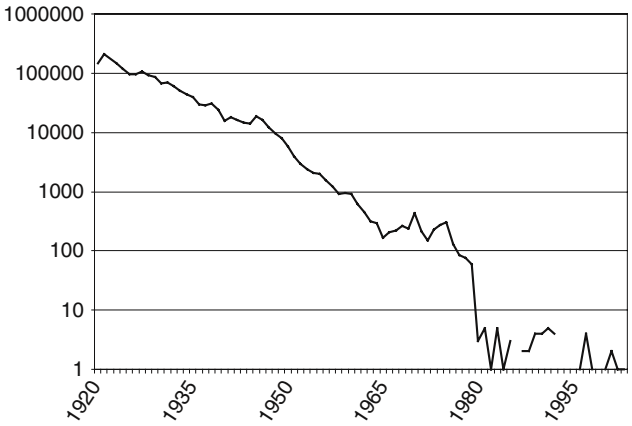


Figure 1. Diphtheria, United States, 1920–2004: Reported cases by year. No cases of respiratory disease due to *C. diphtheriae* were reported in 1986, 1993, 1995, 1999, and 2004–2005.

In the United States, the highest number of diphtheria cases reported since national reporting began in 1920 was 206,939 in 1921 (Figure 1), an incidence rate of 191 per 100,000 population. The same year, 15,520 deaths from diphtheria were reported (mortality rate 14.3 per 100,000 population, case fatality ratio 7.5%). Over subsequent decades, diphtheria incidence and mortality rates progressively decreased, while the case fatality rate remained little changed.[8] Lower case fatality ratios during the 1970s reflected the increased proportion of cutaneous diphtheria cases, which are rarely fatal, reported during that period. The last major outbreak of diphtheria in the United States occurred in King County, Washington, from 1972 to 1982.[70] Of 1,100 cases identified during the outbreak, 947 (86%) were cutaneous infections. From 1980 to 2005, a total of 51 cases of diphtheria due to infection with *C. diphtheriae* were reported in the United States, of which 20 (39%) were confirmed by culture (Figure 2). An additional three cases of respiratory disease due to infection with toxigenic strains of *C. ulcerans* also were reported. Although diphtheria is a rare disease in the United States, endemic foci have been documented in communities in both the United States and Canada.[39,40,73] In less developed nations, diphtheria vaccination was introduced with the expanded program on immunization (EPI) in the late 1970s. Before this, it was estimated that close to 1 million cases of diphtheria occurred annually in developing countries, with 50,000–60,000 deaths.[74] Until the 1990s, reported diphtheria was decreasing in all regions, and eradication of diphtheria was considered possible.[75] However, during the 1990s, epidemic diphtheria returned to the new independent states of the former Soviet Union. As few as 198 cases (0.08 per 100,000 population) were reported in the entire Soviet Union in 1976; in 1989, 839 cases. The epidemic began in Russia in 1990, spread to Ukraine in 1991, and in 1993–1995 spread to the other new independent states. At the height of the epidemic, in 1994–1995, almost 100,000 cases were reported in the new independent states. With aggressive implementation of immunization programs aimed at both children and adults, the outbreak came under control in the late 1990s.[76] A hypothesis to explain why a dramatic drop in the incidence of diphtheria has followed use of a vaccine that induces immunity only to the *tox* gene of *C. diphtheriae* has been advanced by Pappenheimer.[3] The *tox* gene is not essential to the growth of the organism, but likely confers upon it a selective advantage in nonimmune populations. The toxin, by producing tissue necrosis and a site for greater replication of the bacterium in diphtheria cases, facilitates its spread to other susceptible persons. However, in an immune population, infection with toxigenic *C. diphtheriae* does not lead to clinical disease, and this selective advantage is lost, which leads to a reduction in the circulation of toxigenic *C. diphtheriae*. Pappenheimer cites data from Romania in support of this theory. Routine diphtheria immunization began in Romania in 1958, and within several years, the percentage of Schick-negative (immune) individuals in the population had increased from 60 to 97%. The incidence of diphtheria fell rapidly to very low levels. Analysis of *C. diphtheriae* isolates from carriers revealed that, lagging behind the fall in diphtheria incidence, the proportion of strains that were toxigenic fell from 87% in 1958 to only 4% in 1972. The low incidence of diphtheria observed in countries that have maintained high immunization coverage among children is consistent with Pappenheimer's hypothesis, but data on the prevalence of carriage with either toxigenic or nontoxigenic *C. diphtheriae* are limited.

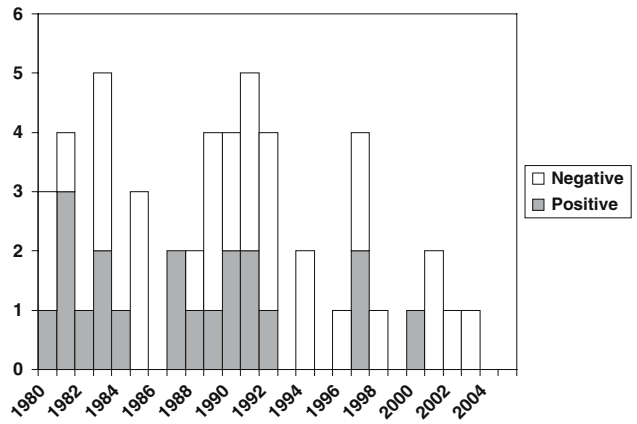


Figure 2. Respiratory diphtheria, United States, 1980–2005. Cases by year and culture status. Data for 1980–1995 from ref. 9.

5.2. Epidemic Behavior and Contagiousness

From at least the 15th century in Europe, diphtheria occurred in epidemics of varying virulence. During some periods there appeared to be a 12- or 15-year periodicity to these outbreaks, but there were also intervals of several decades with little reported diphtheria. Diphtheria incidence increased in the latter part of the 19th century in Europe and the United States, with high case fatality rates.[77] There were significant increases in diphtheria incidence during World War II in many countries, which has been attributed to dissemination of *C. diphtheriae* by military personnel returning from desert or tropical areas.[78,79] In the vaccine era, outbreaks, usually quite limited in geographic extent, have continued to occur in industrialized countries, but with decreasing frequency. However, the epidemic which occurred in the former Soviet Union represented a significant reversal in this trend.

Both asymptomatic carriers and clinical cases are important in the spread of diphtheria. In household settings, up to 27% of close contacts of cases may be found to be infected.[80] Transmission from cases is more efficient than from asymptomatic carriers, presumably because of the greater number of infecting organisms (and perhaps certain features of the infecting organism). Secondary cases are at least 10 times more common after household exposure to cases compared to household exposure to carriers.[81] Although carriers are less infectious than cases, in diphtheria endemic areas, carrier prevalence is many times higher than the prevalence of cases; therefore, carriers have a greater role in transmission. This problem is exemplified by noting that during the first decades of the 20th century, 2–5% of schoolchildren in urban areas were carriers of toxigenic *C. diphtheriae*.[72,81]

5.3. Geographic Distribution

In the prevaccine era, diphtheria had a worldwide distribution. In temperate regions, respiratory diphtheria predominated, with cutaneous diphtheria being relatively uncommon. By contrast, in many (but not all) tropical and desert regions, cutaneous diphtheria was the usual form of the disease, with little or no respiratory diphtheria reported despite moderate rates of pharyngeal carriage of *C. diphtheriae*. In these countries, intense skin transmission appears to occur in the first few years of life, resulting in development of immunity to diphtheria toxin.[82–84]

Since the 1980s, a state of near elimination of toxigenic *C. diphtheriae* has existed in many industrialized countries. However, with the extent and rapidity of travel today, there is a continuing potential for outbreaks in such countries following importation of toxigenic strains from countries where diphtheria is still occurring.[85,86,87] Such an event was suspected as the reason for a series of outbreaks, with 33 cases of respiratory diphtheria, that occurred in Sweden in the mid-1980s, after the absence of indigenous diphtheria for 20 years.[31,32] Additionally, foci of endemic transmission of *C. diphtheriae* have been identified in both the United States and Canada.[39,40] Thus, clinicians, public health authorities, and laboratorians all must remain vigilant, in spite of low incidence now seen in many countries.

5.4. Temporal Distribution

Diphtheria has a marked seasonal variation in incidence in temperate countries. Incidence peaks in the autumn and early winter and is at its lowest in late spring and early summer.[88]

5.5. Age

Until the 1970s, respiratory diphtheria was a disease of children. The highest attack rates were among preschool children and schoolchildren aged 5–9 years. Eighty to ninety percent of cases were among children aged less than 15 years.[72] However, in the vaccine era, there has been a shift in the age distribution toward adults.[89] In the United States from 1980 to 1995, 27 (66%) of the 41 reported diphtheria cases were among persons aged over 20 years.[9] In the recent outbreak in the former Soviet Union, a majority of reported cases were among adolescents and adults, although the proportion of cases by age group varied among the countries.[76] This change in age distribution is explained by the fact that children are mostly protected by immunization, whereas many adults are now susceptible, because exposure to toxigenic *C. diphtheriae* has become rare and vaccine-induced immunity wanes over time unless periodic booster doses of diphtheria toxoid are given. Susceptibility may be particularly high among those cohorts who were children during the early years of diphtheria immunization program, who may have grown up during a period of decreased diphtheria incidence but not all children were vaccinated. This presumably accounts for the severity of the disease and high case fatality rate for adults 30–50 years of age that was observed in the former Soviet Union.[76]

5.6. Sex

There does not appear to be a particular susceptibility of either gender to clinical diphtheria. If gender-specific differences in incidence occur, they probably reflect differences in levels of exposure of *C. diphtheriae*. During the outbreak

in the former Soviet Union in the 1990s, women had higher incidence rates than did men in those areas where the sex distribution of cases was assessed.[76] In Ukraine, disease incidence was similar between boys and girls <10 years of age, but among older adolescent girls and women, incidence rates were higher than those observed among males.[90] In Kazakhstan, 63% of adult case-patients were female,[91] and in Moldova, incidence rates were 2- to 3-fold higher among women than among men.[92] These differences are thought to reflect greater contact by older adolescent and adult females with children, compared to males.

5.7. Race

In the United States during the period 1959–1970, the incidence rate among Native Americans was 3 times higher than persons of black race and 20 times higher than persons of white race.[7] From 1971 to 1981, incidence rates of respiratory diphtheria were approximately equal in whites and blacks, but rates in Native Americans were 100 times higher.[6] A strikingly higher incidence of diphtheria, particularly of the skin and ear, among Native American populations has also been noted in Canada.[93] Endemic circulation has been documented in a Native American community in South Dakota.[73] A higher incidence of diphtheria, in comparison to whites, has also been reported in Australian aborigines.[94] These racial differences likely represent mostly differences in living conditions (e.g., increased contact rates and reduced hygiene) that promote transmission of diphtheria, particularly in its cutaneous form.

5.8. Occupation

Medical workers exposed to diphtheria in the prevaccine era were reported as having developed higher rates of carriage of the organism compared to the general population.[72] Although cases of clinical diphtheria did occur among medical personnel, frequently the result was asymptomatic infection, since high levels of immunity were maintained by continuing exposure to diphtheria patients. More recent studies of hospital staff contacts of diphtheria cases indicate a low risk of acquisition of the organism.[80]

Diphtheria outbreaks have frequently been reported among military personnel, particularly when deployed in tropical or desert settings, and returning soldiers can import diphtheria into civilian populations.[83,95–97] The Soviet military appears to have played a role in the recent epidemic of diphtheria in the former Soviet Union.[98,99] Military personnel are at increased risk of diphtheria because of crowded living conditions and, especially in combat situations, poor hygiene and frequent occurrence of skin lesions, which can become secondarily infected with *C. diphtheriae*.

5.9. Occurrence in Different Settings

The most recent outbreaks in the United States and Western Europe have characteristically involved innercity, indigent, homeless, and/or alcoholic persons (so-called "skid road" inhabitants).[31,43,70] Such persons were also said to experience an increased risk of diphtheria in the recent outbreak in the former Soviet Union.

Institutional outbreaks have occurred in mental hospitals (where the clinical diagnosis of diphtheria may be delayed), day care centers, and schools.[80,97,100–102] Schools and preschool institutions have traditionally been an important setting for transmission among children.[81,100,103–104]

5.10. Socioeconomic Factors

Low socioeconomic status is a risk marker for diphtheria, to the extent that it is associated with crowded living conditions and reduced hygiene and with reduced immunization levels.

6. Mechanism and Routes of Transmission

The organism is carried in the nose and throat[105] and appears to have a particular predilection for tonsillar tissue.[106,107] It also readily infects cutaneous lesions. Transmission occurs in close-contact settings via respiratory droplets or by direct contact with respiratory secretions or skin lesions. The organism survives on clothes and dust in the environment of cases, and fomite transmission via items such as clothes and towels may be important, particularly in settings of crowding and poor hygiene.[50,108] Compared to respiratory carriers, persons with skin lesions infected with *C. diphtheriae* (which frequently are unrecognized) apparently contaminate the environment with the bacterium and transmit it more efficiently to both the skin and respiratory tract of household and school contacts.[50,109,110] However, certain persons with nasopharyngeal infection—both cases and carriers—have been described as "spreaders" because of their apparently efficient dissemination of the organism. *C. diphtheriae* can be readily cultured from the upper lip, hands, and clothes of such individuals.[108] Foodborne transmission, most often by infected milk or milk products, has been occasionally implicated.[45,111,112]

7. Pathogenesis and Immunity

In classical respiratory diphtheria, *C. diphtheriae* colonizes the mucosal surface of the nasopharynx and multiplies locally without bloodstream invasion. The incubation period is from 1 to 7 days (usually 2–5 days). Released toxin causes local tissue necrosis and tough, adherent pseudomembrane forms, composed of a mixture of fibrin, dead cells, and bacteria. The membrane usually begins on the tonsils or posterior pharynx. In more severe cases it spreads, extending progressively over the pharyngeal wall, fauces, soft palate, and into the larynx (and on occasions further into the trachea and bronchi), resulting in respiratory obstruction. Toxin entering the bloodstream causes damage at distant sites, particularly the heart (myocarditis), nerves (demyelination), and kidney (tubular necrosis).

Primary infection of the skin may occur, which classically results in a "punched out" ulcer, often with a grayish membrane. However, more frequently *C. diphtheriae* appears to secondarily infect preexisting skin lesions.

Diphtheria toxin, a two subunit protein with a molecular weight of 61,000 kDa, is an extremely potent inhibitor of protein synthesis, with an estimated human lethal dose of 0.1 μg/kg body weight. The B subunit attaches to the cell surface. The A subunit enters the cell and, by ADP ribosylation, inactivates elongation factor 2, which is an essential factor in the transfer of amino acids to the nascent polypeptide chain.[3,113] The extent of toxin absorption varies with the site of infection, being much less from skin or nose than from the nasopharynx.

Immunity to clinical diphtheria appears largely to depend on the level of diphtheria antitoxin in the serum at the time of onset of illness (Section 3.2.2). Antibodies to fragment B of diphtheria toxin are able to neutralize the effect of toxin and protect against disease. Diphtheria antitoxin is predominantly IgG antibody.[114] It is transmitted transplacentally, providing protection to infants during the first 6 months of life.

Stimulation of antitoxin production occurs with either asymptomatic infection or disease, in addition to immunization with diphtheria toxoid. Following recovery from clinical diphtheria, development of protective immunity is not reliable[115] and vaccination is recommended in convalescence. The possession of protective levels of antibody does not prevent carriage of *C. diphtheriae* nor does it appear to affect the duration of carriage. On the contrary, identified carriers may have relatively high levels of antitoxin.[43]

Waning of the diphtheria antitoxin level occurs over time, following either natural infection or artificial immunization. Periodic booster doses of diphtheria toxoid are recommended to maintain protective levels of antitoxin. Although a boost in antitoxin levels may occur following the onset of disease in individuals who have been previously immunologically primed but whose antitoxin concentration has fallen below the protective level, in many cases the production of diphtheria toxin by the infecting organism is so overwhelming that the antitoxin response is not swift enough to prevent complications or even death.

8. Patterns of Host Response

8.1. Clinical Features

8.1.1. Carrier State. Asymptomatic nasopharyngeal infection with *C. diphtheriae* occurs more frequently than clinical disease. The duration of carriage was studied by several investigators during the preantibiotic era, by examining the rate of disappearance of *C. diphtheriae* from the nasopharynx of either convalescent cases or identified carriers. Hartley and Martin[106] followed 457 cases and found that 49, 74, and 87%, respectively, were culture-negative by 15, 30, and 45 days after illness onset. Weaver[116] studied the rate of disappearance of *C. diphtheriae* among 500 cases. After the first week, approximately half of the cases that began the week culture-positive became culture-negative during the following 7 days. By 3 weeks after onset, 71% had become culture-negative; by 4 weeks, 83%; and by 8 weeks, 99%. Weaver also studied the rate of disappearance of *C. diphtheriae* in 52 carriers: by 2 weeks after identification, 55.8% had become culture-negative and by 4 weeks, 80.8%. Two (4%) remained positive after 75 days. Differences between the results of these studies probably largely reflect the greater opportunity for reinfection of Hartley and Martin's patients. Both authors describe the association of chronic carriage with large, irregularly shaped tonsils and the value of tonsillectomy in eradicating carriage. However, this should not be necessary with the present availability of effective antibiotics.

8.1.2. Respiratory Diphtheria. Infection limited to the anterior nares manifests as chronic serosanguinous or seropurulent discharge, without fever or significant toxicity. A whitish membrane may be observed on the septum. Such patients may readily spread diphtheria. The faucial (pharyngeal) form is the most common. The illness begins insidiously with a sore throat, malaise, and mild to moderate fever. There is an initial mild pharyngeal erythema, usually followed by progressive formation of a whitish tonsilar exudate, which over 24–48 hr changes into a gray adherent membrane, which is tightly adherent and bleeds upon attempted removal. In more severe cases, the patient appears toxic and the membrane is more extensive. Gross

enlargement of cervical lymph nodes and surrounding tissue edema may occur, resulting in the so-called "bull neck." Laryngeal involvement, which may occur on its own or as a result of membrane extension from the nasopharynx, presents with hoarseness, stridor, and dyspnea. Intubation or tracheostomy may be required.

The likelihood of toxic complications depends on the severity of disease at presentation and the interval between disease onset and administration of therapeutic antitoxin. Myocarditis typically occurs between 1 and 2 weeks after the onset of respiratory symptoms. Neural impairment occurs in 10–20% of patients. In severe cases, palatal and/or pharyngeal paralysis may occur during the acute phase. Cranial nerve involvement may develop after the first week. Peripheral neuritis, symmetrical and predominantly motor, occurs from 2 to 12 weeks after disease onset. Motor deficit may range from minor proximal weakness to complete paralysis. Although slow (several months), recovery of neurological function is usually complete. In fulminant, sometimes called hypertoxic, diphtheria, toxic circulatory collapse with hemorrhagic features occurs. More severe disease occurs in the very young and the elderly and in unimmunized persons.

8.1.3. Cutaneous Diphtheria. Classical cutaneous lesions are indolent deep punched out ulcers, which may have a grayish-white membrane. More commonly, however, *C. diphtheriae* appears as a secondary infecting agent of a variety of dermatoses such as traumatic lesions, pyoderma, or chronic dermatitis, such as stasis dermatitis.[70,109] There is frequently coinfection with *Streptococcus pyogenes* and/or *Staphylococcus aureus*. Recognized toxin-induced diphtheria complications are usually stated to be uncommon, which is said to be because toxin absorption from the skin is much less than from the nasopharynx. However, toxin-induced neuritis and myocarditis were noted following cutaneous diphtheria among soldiers during World War II.[83,95]

8.1.4. Other Sites. The organism may uncommonly infect other sites, including the eye, genitalia, and ear.

8.1.5. Invasive Disease. Uncommonly, *C. diphtheriae*, both toxigenic and nontoxigenic, may cause invasive disease, including endocarditis, osteomyelitis, septic arthritis, and meningitis.[54–59] Frequently, but not always, these patients have predisposing factors such as a prosthetic cardiac valve or underlying immunosuppression.

8.2. Diagnosis

The decision to initiate therapy should be made on clinical grounds, since delay in treatment is associated with worse outcomes, owing to the fact that antitoxin can only neutralize toxin that has not already bound to cells. A high index of suspicion is required. Cultures should be taken from beneath the membrane, from the nasopharynx, and from any suspicious skin lesions. Because special media are required, the microbiology laboratory should be alerted to the concern about diphtheria. Cultures may be negative if the patient received previous antibiotics. The diagnosis of diphtheria is more likely if the patient is unimmunized or has not received recommended booster immunizations; if there is a history of contact with a known diphtheria case; if there is a history of recent travel to a region with endemic diphtheria or of contact with a recent immigrant from such an area; if the patient belongs to a population group that has had a higher risk of diphtheria in the past, such as homeless persons; and if the pretreatment antitoxin titer is <0.01 IU/ml.

The differential diagnosis of respiratory diphtheria includes bacterial (streptococcal, *Arcanobacterium hemolyticum*[117]) and viral tonsillopharyngitis, infectious mononucleosis, peritonsillar abscess, Vincent's angina, candidiasis, and acute epiglottitis. A nasal foreign body produces a unilateral nasal discharge that can mimic nasal diphtheria.

8.3. Treatment

The mainstay of therapy is equine diphtheria antitoxin, which should be administered as the diagnosis is reasonably suspected, without waiting for bacteriologic confirmation, in order to neutralize toxin before it binds to tissues. Antibiotic therapy (penicillin or erythromycin) is necessary to eliminate the organism, thereby halting further toxin production and preventing further transmission.

9. Control and Prevention

9.1. General Concepts

Recommendations for the prevention and control of diphtheria, including an algorithm to guide investigation and management, have been published by the CDC and by the WHO Regional Office for Europe.[80,118]

The important first step is recognition of suspected diphtheria by clinicians. Although most practicing clinicians have never seen a case of the disease, it is important that they remain aware of the possibility of diphtheria, particularly in a patient with a nasopharyngeal pseudomembrane. Identification of a suspected case should set in motion a series of activities. The health department should be notified so that the contact investigation can begin as soon as possible. The patient is placed in strict respiratory isolation, the laboratory notified of the suspicion of

diphtheria, and the appropriate cultures taken, if possible before antibiotics are begun. During and after treatment, isolation should be maintained until elimination of the organism is demonstrated by two negative cultures obtained at least 24 hr apart after completion of antimicrobial therapy. The patient should be immunized with diphtheria toxoid during convalescence.

Close contacts (which include household contacts and others spending at least several hours per week in close proximity to the patient, such as preschool and elementary school classmates), irrespective of vaccination status, should be (1) monitored for symptoms and signs of diphtheria for at least 7 days, (2) cultured from the nose and throat for *C. diphtheriae*, and (3) given antibiotic prophylaxis (Section 9.2). The immunization status of all close contacts should be assessed and diphtheria toxoid administered as indicated. Persons who have not previously received at least three doses of diphtheria toxoid should receive a dose of diphtheria toxoid and then further doses as necessary to complete the recommended schedule for age. The schedule can be completed irrespective of the interval between the previous doses. Persons who have received three or more previous doses of diphtheria toxoid should receive a further dose unless their last dose was received within the past 5 years. Contacts found to be carriers of *C. diphtheriae* should receive follow-up cultures a minimum of 2 weeks after completion of antibiotics, to ensure eradication of the organism.

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis

Although the effectiveness of antibiotic prophylaxis in preventing secondary cases among contacts is not proven, it is recommended[80,118,119] that all close contacts should receive antibiotic prophylaxis, after appropriate cultures have been taken. The importance of antibiotic treatment of carriers, in addition to immunization, in the control of diphtheria has been reinforced by experience during outbreaks in the United States.[100,103] If compliance is assured, a 7- to 10-day course of erythromycin (40 mg/kg per day for children and 1 g/day for adults, in divided doses) is recommended; if compliance is doubtful or if there is intolerance to erythromycin, a single intramuscular injection of benzathine penicillin (600,000 units for persons <6 years of age and 1.2 million units for persons ≥ 6 years of age) is preferred. Both clinical and laboratory data indicate that erythromycin may be more effective than penicillin.[120,121]

9.3. Immunization

Both theoretical data and the occurrence of outbreaks in even highly immunized communities[100,103,122,123] suggest that a high level of population immunity, perhaps greater than 85%, is necessary to prevent and to control outbreaks. This contrasts with earlier opinions (offered at a time when levels of natural immunity among adults were high) that diphtheria could be controlled if childhood immunization rates of 70% were achieved.[41] Now that natural exposure to toxigenic *C. diphtheriae* is rare, the necessary levels of immunity can only be realized by achieving childhood vaccination coverage of at least 95% and by achieving high coverage of adults with periodic booster doses of diphtheria toxoid. In 1999, 42% of adults 65 years of age and older reported receiving tetanus-containing vaccine (almost all of which likely included diphtheria toxoid, as adult formulation diphtheria and tetanus toxoids) within the previous 10 years. Coverage was higher among those aged 50–64 years (57%) and 18–49 years (66%).[124] Immunization with diphtheria toxoid is the mainstay of diphtheria prevention. Almost always, diphtheria toxoid is administered to children in vaccines in which it is combined with one or more other antigens (tetanus toxoid with or without pertussis vaccine [whole cell or acellular]: DT, DTP, and DTaP, respectively). Combination vaccines in which DTaP is additionally combined with inactivated polio vaccine (IPV) and hepatitis B vaccine, or IPV and *Haemophilus influenzae* type B conjugate vaccine are also available. Pediatric vaccines usually contain from 10 to 30 Lf (flocculation units) of diphtheria toxoid. For persons aged 7 years of age or older, adult formulation tetanus and diphtheria toxoids (Td) are used for both primary and booster immunization. The small "d" reflects a reduced amount of diphtheria toxoid (in many countries, approximately 2 Lf) in order to reduce adverse effects, particularly severe local reactions, experienced by some older individuals upon receipt of higher amounts of diphtheria toxoid.[125,126]

Three different vaccination schedules are shown in Table 1. Issues relating to the immunogenicity of different schedules have been reviewed elsewhere.[127] For both adults and children, three doses of diphtheria toxoid are required to raise antitoxin levels above the minimum protective level (0.01 IU/ml) in 100% of recipients.[127,128] In the absence of ongoing natural exposure to toxigenic *C. diphtheriae*, vaccine-induced immunity wanes over time.[129–133] Periodic diphtheria toxoid booster doses are therefore required to maintain immunity: in the United States, decennial boosters are recommended. In some tropical countries, ongoing exposure to cutaneous diphtheria may reduce the need for diphtheria toxoid boosters[127,134]; however, persons in

Table 1. Schedules for Diphtheria Vaccination Recommended in the United States, in the United Kingdom, and by the World Health Organization Expanded Program on Immunization

Dose	United States (ACIP, AAP)[141]		United Kingdom[142]		WHO, EPI[143]	
Primary						
1	2 months	DTaP	2 months	DTaP	6 weeks	DTP
2	4 months	DTaP	3 months	DTaP	10 weeks	DTP
3	6 months	DTaP	4 months	DTaP	16 weeks	DTP
4	12–18 months	DTaP	–		–	
Booster						
1	4–6 years	DTaP	3 years 4 months	DTaP or Tdap		
2	11–12 years	Tdap	–			
3	Every 10 years	Td				

Abbreviations: ACIP, Advisory Committee on Immunization Practices; AAP, American Academy of Pediatrics; WHO EPI, World Health Organization Expanded Program on Immunization.

low-endemic or non-endemic areas may require boosters at about 10-year intervals to maintain immunity.[135]

The most frequent adverse reaction to diphtheria toxoid is local pain at the site of inoculation, sometimes with associated redness and swelling and occasionally severe. Systemic reactions (fever and malaise) occur infrequently. Most such reactions are believed to represent hypersensitivity reactions to diphtheria toxoid or to associated impurities.[126] The assessment of adverse reactions is difficult because diphtheria toxoid is now almost always administered in combination with tetanus toxoid and other antigens; however, available data suggest that addition of diphtheria toxoid to tetanus toxoid results in more adverse reactions than are seen following tetanus toxoid alone.[135–138] Severe generalized anaphylactic reactions rarely have been reported.[139]

The effectiveness of diphtheria toxoid has never been measured in a controlled trial. Its value seemed obvious to early investigators, and the Schick test and serological methods provided measurable correlates of protection. However, studies comparing the incidence of diphtheria in vaccinated and unvaccinated individuals and case–control studies have provided estimates of effectiveness of 70–98%.[140] While immunization with diphtheria toxoid does not provide complete protection against clinical diphtheria, when disease occurs in fully vaccinated persons, it is generally milder and is rarely fatal.

10. Unresolved Problems

In this era of apparent near elimination of toxigenic *C. diphtheriae* from most developed nations, diphtheria is often considered a disease of the past, and therefore interest in research on this disease has waned, with the exception of ongoing molecular studies related to diphtheria toxin. Several issues related to nontoxigenic *C. diphtheriae* strains deserve study. The current epidemiology of nontoxigenic organisms is poorly understood. This information is potentially important, since the introduction, through an imported toxigenic strain, of a tox^+ phage into the resident pool of nontoxigenic organisms is more likely if they continue to circulate widely. Nontoxigenic organisms may cause local disease, at times severe; in addition, severe local disease due to toxigenic strains may occasionally occur in persons with high levels of diphtheria antitoxin. A better understanding of the pathogenesis of disease caused by nontoxigenic organisms may assist in developing future vaccines that both provide more certain protection against disease and also protect against carriage (asymptomatic infection).

There are surprisingly few published studies from which to establish the current recommendations for prophylaxis of contacts of diphtheria cases and treatment of carriers of *C. diphtheriae*. The most important issue, however, is the necessity to maintain vigilance (i.e., surveillance) to ensure that *C. diphtheriae* will not reemerge in those countries where it is now apparently absent. This requires maintaining the clinical and laboratory capacity to recognize and diagnose diphtheria and, crucially, improving levels of immunity among adults by increasing awareness among both the public and medical providers of the importance of regular tetanus–diphtheria boosters.

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CHAPTER 15

Escherichia coli Diarrhea

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1. Introduction

During the 1940s and 1950s, a series of outbreaks of diarrhea in hospital newborn nurseries were reported in which the etiologic agent appeared to be *Escherichia coli* identified by serotype. These strains became known as enteropathogenic *E. coli* (EPEC). Since the Second International Symposium on EPEC in 1995, EPEC strains have been renamed typical EPEC (tEPEC) to distinguish them from another not well characterized *E. coli* strains known as atypical EPEC (aEPEC).[1] In this chapter we will focus on tEPEC although some general characteristics of aEPEC will be also mentioned.

Since the 1970s and 1980s, enterotoxigenic *E. coli* (ETEC) strains have been shown to be important causes of diarrhea among children living in developing countries and among international visitors to the areas (travelers' diarrhea). ETEC strains are being introduced into developing regions such as the United States.

Enteroinvasive *E. coli* (EIEC) strains have been identified in patients with dysenteric illness clinically indistinguishable from that produced by *Shigella* strains.

Enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) strains were shown to cause colitis with dysentery and to produce hemolytic uremic syndrome primarily in children. While the most common serotype of STEC is O157:H7, many other serotypes of shigatoxigenic *E. coli* can cause illness. Our food supply is currently an important source of STEC infection.

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E. coli strains showing enteroaggregative attachment to epithelial cells and referred to as enteroaggregative *E. coli* (EAEC) were identified as causes of persistent diarrhea in infants living in developing countries, AIDS-associated diarrhea and travelers' diarrhea. EAEC strains have been implicated as causes of pediatric diarrhea in the United States.

There is a sixth potential classification of diarrheagenic *E. coli* showing diffuse attachment to tissue culture cells. These so-called diffusely adherent *E. coli* (DAEC) are currently being evaluated in cases of diarrhea. The pathogenicity of these strains is currently uncertain. DAEC will not be included in this review.

In this chapter, we will review information available about EPEC, ETEC, EIEC, STEC and EAEC. Since the organisms are biochemically similar—each represents an *E. coli*, they will be discussed in the same sections even though their clinical features and epidemiology are distinctly different.

2. Historical Background

In the 1940s serotype-identified *E. coli* were shown to cause nursery outbreaks of diarrhea. EPEC strains have since been shown to have a worldwide distribution. Serological procedures were first developed to define these EPEC strains according to their somatic (O) and flagellar (H) antigens; however, the strains are best defined on the basis of pathogenic characteristics of intestinal attachment and effacing histopathology.

Taylor et al.[2] reported in 1961 that *E. coli* isolated from stools of a number of children with a diarrheal syndrome caused dilatation from transudation of fluid and electrolytes in rabbit ileal loop preparations, while strains recovered from healthy infants, from well water or from the urine of patients with urinary tract infections produced a negative reaction. In

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Table 1. Important *E. coli* Serotypes

EPEC	ETEC	EIEC	STEC	EAEC
O18:H7	O6:H16/$^-$	O28:H$^-$	O4:H$^-$	O3:H2
O20:H26	O8:H9/$^-$	O112:H$^-$	O5:H$^-$	O7:H$^-$
O25:H1	O15:H11/$^-$	O115:H$^-$	O16:H6	O15:H18
O26:H11/$^-$	O20:H$^-$	O124:H30/32/$^-$	O26:H11/21/32	O44:H18
O44:H34	O25:H42/$^-$	O136:H$^-$	O46:H31	O77:H18
O55:H6/7/$^-$	O27:H7/20	O143:H$^-$	O48:H21	O86:H$^-$
O86:H27/34/$^-$	O63:H12 or H$^-$	O144:H$^-$	O55:H7	O111:H21
O91:H7/$^-$	O78:H11/12/20	O147:H$^-$	O91:H10/21	O126:H27
O111H2/12/$^-$	O85:H7	O152:H$^-$	O98/H$^-$	O127:H2
O114:H10/32	O115:H11/40	O159:H$^-$	O111:H2/8/$^-$	
O119:H6/$^-$	O128:H7/21	O164:H$^-$	O113:H21	
O125:H21/$^-$	O148:H28	O167:H4/5/$^-$	O117:H14	
O126:H2/$^-$	O149:H10		O118:H12	
O127:H9/21/40/$^-$	O159:H20		O119:H6	
O128:H2/7/8/12/$^-$	O148:H28		O125:H$^-$	
O142:H6	O167:H5		O126:H8	
O158:H23	O173:H$^-$		O128:H2	
			O145:H$^-$	
			O157:H7/$^-$	
			OO172:H$^-$	

EPEC, enteropathogenic; ETEC, enterotoxigenic; EIEC, enteroinvasive; EHEC, enterohemorrhagic; EAEC, enteroaggregative. Multiple H types found in each category are indicated (e.g., H2/7/8/12/$^-$ indicates that H2, H7, H8, H12 and H$^-$ [non-motile] strains have been identified with the O group provided)

1970, an O148 strain of *E. coli* was reported to have produced diarrhea in British soldiers stationed in the Middle East. A closely related O148 *E. coli* strain was later shown to be enterotoxigenic causing diarrheal illness resembling mild cholera in volunteers.[3] In 1970, two different enterotoxins separated by heat susceptibility were shown to be produced by *E. coli* pathogenic for swine.[4] Colonization fimbriae were shown to confer an ETEC adherence capacity for epithelial cells.[5] ETEC strains have been shown to be the most important cause of diarrhea among international travelers from highly industrialized regions to developing countries.

EIEC strains were first demonstrated as causative agents of diarrhea in Japan[6] and Brazil.[7] The similarity of these strains to *Shigella* in causing bacillary dysentery was demonstrated in volunteer studies.[3] Widespread outbreaks of EIEC diarrhea have occurred in the United States[8–10] and other countries associated with contaminated food. EIEC strains have been shown to have a low level of endemic occurrence in cases of diarrhea in nearly all parts of the world. Similarly pathogenic features of EIEC diarrhea are almost identical to those of *Shigella* such as invasion of the colonic epithelium, intracellular multiplication and extension into adjacent epithelial cells.

STEC strains have emerged as a distinctive *E. coli* pathotype following two independent epidemiological observations in 1982 and reported in 1983. Riley et al.[11] reported two large gastrointestinal outbreaks in the United States associated with consumption of undercooked hamburgers served at "fast-food" restaurants. The outbreaks were characterized by occurrence of hemorrhagic colitis (severe abdominal pain and watery diarrhea followed by grossly bloody diarrhea with little or no fever) where stool cultures grew *E. coli* serotype O157:H7 that was not invasive or enterotoxigenic. The second report by Karmali et al.[12] described an association of sporadic case of hemolytic uremic syndrome (triad of acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia) with enteric infection by an *E. coli*-producing cytotoxin activity on Vero cells (verotoxin). While the most common serotype of STEC strains is O157:H7, O26:H11 and many other serotypes may be identified in the enteric disease caused by STEC strains (see Table 1).

In 1985 HEp-2 tissue culture-adherent *E. coli* were found in an important percentage of US travelers to Mexico with diarrhea[13] helping to explain the observation that antibacterial drugs were beneficial in shortening travelers' diarrhea in those with otherwise pathogen-negative illness.

3. Methodology

3.1. Sources of Mortality Data

Little is known about the actual incidence and mortality of illness seen with diarrheagenic *E. coli* infection. It is necessary to rely on outbreak data and information obtained

from prevalence surveys. The mortality of EPEC diarrhea from earlier studies varied widely from 0 to as high as 70%. The average mortality rate has been reported to be 5–6%. An increased mortality, reaching 16%, has been seen in newborns particularly if illness is complicated by prematurity. EPEC strains appear to differ in virulence characteristics. *E. coli* O111 was shown to be associated with a striking clinical illness resulting in the common occurrence of fatalities.[14]

ETEC strains produce self-limiting disease in healthy persons from the industrialized countries traveling to most regions of the developing world, but severe cholera-like illness can occur.[3,15] Mortality is unusual with ETEC, but it undoubtedly occurs in areas of the world where health standards are low and where fluid and electrolyte replacement is not established in those with dehydrating illness. There are no accurate data defining expected EIEC mortality rates, but reported studies suggest that deaths would be unusual.

Uncomplicated STEC infection including dysenteric disease shows a low mortality rate. In a comprehensive community outbreak of O157:H7 infection, 3 of 501 (0.6%) cases died.[16] When complicated by the hemolytic uremic syndrome, STEC diarrhea is associated with a mortality rate of approximately 10%.

Enteroaggregative *E. coli* (EAEC) is an unusual cause of death with fatalities occurring primarily in patients with advanced AIDS.

3.2. Sources of Morbidity Data

Since *E. coli* diarrhea other than that caused by STEC is not a reportable disease, data on morbidity must come from survey information.

3.3. Surveys

Sporadic cases and community and hospital nursery outbreaks of EPEC diarrhea have been documented in nearly every country where appropriate studies have been carried out. Three types of studies have been done to determine the importance of EPEC illness: surveys of the relative frequency of recovering EPEC strains in populations with diarrhea; study of newborn nursery outbreaks of EPEC disease; and surveillance of EPEC strains during short periods in communities under study. While it is impossible to state the prevalence and incidence of diarrhea with accuracy, in approximately 10% of diarrhea episodes in infants and young children an EPEC strain can be recovered from stools.

Surveys of ETEC, EIEC, STEC and EAEC diarrhea have been restricted to short-term studies of diarrhea cases and outbreaks in several regions of the world.

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of Organisms. The methodology for *E. coli* isolation is readily available in microbiology texts, and any diagnostic laboratory that performs bacteriological isolation can isolate *E. coli* with proficiency. Biochemically, diarrheagenic *E. coli* are not distinct from the nonpathogenic *E. coli* present normally in the intestine. *E. coli* strains that cause illness can only be differentiated from normal flora strains by the demonstration of a virulence property. This has always presented a problem for the routine clinical bacteriology laboratory because demonstration of *E. coli* virulence properties or *E. coli* pathotypes requires methodology generally available only in research and reference laboratories. This diagnostic problem has limited what we know about the epidemiology of *E. coli* diarrhea, although recent advancements in molecular techniques have greatly improved our diagnostic capabilities.

Complete serotyping of *E. coli* strains requires a determination of both the O and H antigen types. There are approximately 164 O groups and 57 H groups. Table 1 lists the O (somatic) and H (flagellar) serotypes identified with EPEC strains. Virulence assays have largely replaced serotyping for the identification of EPEC strains.

ETEC are diagnosed by the demonstration of the production of one or both of the enterotoxins that they produce. Generally, five *E. coli* colonies per patient-sample need to be tested for both toxins. Immunoassays have been developed for detection of ST, including a radioimmunoassay and an enzyme-linked immunosorbent assay (ELISA). These tests correlate well with results of the sucking-mouse assay and require less expertise. As early as 1982, DNA probes were found to be useful in the detection of LT- and ST-encoding genes in stool and environmental samples.[17] More recently advances in ETEC detection have been made including PCR assays which are sensitive and specific when used directly on clinical samples or on isolated bacterial colonies. A useful adaptation of PCR is the multiplex RCR assay[18] in which several PCR primers are combined with the aim of detecting one of several different diarrheagenic *E. coli* pathotypes in a single assay. Following study with a multiplex PCR, various reaction products can be differentiated by single agent assay.

Presence of a virulent *Shigella* or EIEC strain traditionally has been verified by testing in the guinea pig eye (Sereny) model. In the assay, a heavy suspension of bacteria was dropped into the conjunctival sac of a guinea pig and 1–7 days later observed for presence of purulent keratoconjunctivitis confirming the strain capacity to invade epithelial cells. DNA probes and PCR methods are now available to test directly for the presence of genes encoding for invasiveness.

The presence of STEC O157:H7 strains, the most common serotype in the United States, is determined by isolating sorbitol non-fermenting *E. coli* and verifying the isolated colonies as being O157 positive by agglutination reagents and inhibition of motility in media containing H7 antiserum.[19] Since other serotypes of Shiga toxin-producing *E. coli* may cause illness, either *E. coli* colonies or stool samples should be checked for the production of Shiga toxin by enzyme immunoassay or PCR. If positive for Shiga toxin, they should be sought by culture methods so that sero- and molecular typing can be performed for epidemiologic study.

EAEC strains are identified either using the HEp-2 cell adherence assay or by looking for one or more virulence properties by DNA probe, ELISA or PCR methods.[20, 21] The gold standard for EAEC identification remains the HEp-2 adherence test. This test requires specialized facilities and can therefore only be conducted in reference laboratories. Baudry et al.[22] developed a DNA probe, CVD432, to simplify the identification of strains of EAEC. The CVD432 probe has been shown to have sensitivity for detecting EAEC isolates of between 15 and 99% from various studies carried out in different regions of the world. Tsukamoto[23] proposed a PCR method based on the *aggR* regulatory gene. Although *aggR* is a sensitive target, it may be less specific than the CVD432 locus. The HEp-2 adherence assay and/or the CVD432 standard probe represent the present means for detecting EAEC; however, it is clear that more sensitive, specific and practical methods are needed to improve diagnosis of infected cases and understanding of the disease.

Multiple laboratories are working on the refinement of reagents allowing the detection of diarrheagenic *E. coli* using multiplex polymerase chain reaction (PCR) methods.

In ETEC diarrhea, seroconversion to anti-LT antibody is common but not invariable. An ELISA was developed for the detection of IgG and IgM antibodies to ETEC LT.[24] Anti-LT antibody was associated with previous infection by LT-producing strains of ETEC. The ELISA technique also allows the study of humoral antibody development directed to colonization factor antigens during ETEC infection.

During infection with EIEC, a serological response can be documented.[3] In view of the similarity to bacillary dysentery, most probably the frequency of seroconversion will correlate with the presence of dysentery (passage of small volume bloody mucoid stools), which indicates the colonic mucosal invasion and inflammation.

An ELISA has been developed to detect serum antibodies against *E. coli* O157:H7.[25] This assay has been used along with culture to increase detection of this STEC strain.

Serology has not yet been employed to detect EAEC infection.

4. Biological Characteristics of the Organisms

Volunteer experiments have been conducted to establish the virulence of EPEC strains.[26] EPEC strains have been defined as specific serogroups of *E. coli* that have been epidemiologically incriminated as causes of diarrhea but do not produce conventional enterotoxins and are not invasive. The O:H serogroups recognized as EPEC are listed in Table 1. Biopsies from children infected with an O119 EPEC strain have shown a characteristic lesion consisting of localized adherence of microcolonies, cupping of the enterocyte and destruction of the microvilli.[27] Cravioto et al.[28] developed a tissue culture model for this adherence property using HEp-2 cells. The focal attachment of the strains to small intestinal epithelial cells has been shown to be associated with a pathognomonic lesion at the site of attachment described as attaching and effacing histopathology with microvillus destruction, intimate adherence of bacteria to intestinal epithelium, pedestal formation and aggregation of polarized actin at the site of attachment. Molecular pathogenicity of EPEC infection has been described.[29] There are two chromosomal factors and one plasmid constituent that are essential. The plasmid factor is the bundle forming pilus (*bfp*) that leads to focal attachment of membrane receptors and growth as focal microcolonies. The chromosomal factors are carried on the locus of enterocyte effacement (LEE) and include type III secretory system, *E. coli* secreted protein (Esp), translocated intimin receptor (Tir) that is inserted into the intestinal membrane as a novel organism receptor and the *eae* gene, encoding intimin, which is responsible for the tight epithelial attachment and loss of microvilli (the "attaching and effacing" property). LEE also carries tyrosine kinase which together with Ca^{++} leads to formation of actin-rich pedestals. A DNA probe using *eaf* plasmid fragment is available to screen for EPEC strains as well as PCR primers to identify *bfp* gene. *E. coli* strains that possess the *eae* gene but are negative for the plasmid *bfp* are associated with diarrhea and are known as atypical EPECs.

ETEC strains produce one or both of two enterotoxins, a heat-labile toxin (LT) or a heat-stable toxin (ST). LT resembles cholera toxin, which operates through adenylate cyclase mechanisms, and the second, ST, which is a poorly antigenic, small molecular weight toxin, works through guanylate cyclase stimulation. LT and ST are under plasmid control. ETEC strains may produce LT or ST or both. Some strains that only produce LT show reduced pathogenicity. LT is detected by showing dilatation of a rabbit ileal loop, by showing morphogenic changes in certain cyclic AMP-dependent tissue cultures (adrenal cells, Chinese hamster ovary) and in serologic procedures like in an enzyme-linked immunosorbent assay (ELISA). ST has been traditionally assayed in a suckling mouse model. There exist gene probes,

which can detect genetic material related to the plasmids of LT and ST toxigenicity. Surface protein adhesions, known as colonization factor antigens (CFAs), allow ETEC to attach to intestinal mucosa. One of about 18 defined CFAs are found on the surfaces of between 50 and 80% of ETEC strains found worldwide. Strains of ETEC in a community differ widely in terms of virulence properties suggesting the presence of multiple strains in endemic settings.[30]

EIEC strains possess a *Shigella*-like invasive property that probably represent the important biological activity favoring persistence in nature. This ability to invade epithelial cells is also encoded by genes carried on a plasmid and are the same as those found in *Shigella*. The resemblance of these strains to *Shigella* is further seen in their possession of common antigens. There is a relationship between EIEC and *E. coli* serotype. Table 1 lists the recognized EIEC serotypes. It is unknown why these strains are not more important causes of illness.

The important virulence property of STEC appears to be Shiga toxin (Stx) production. It is not clear if the possession of genes encoding Stx confers pathogenicity. Epidemiological studies have shown an association with disease with STEC strains that actively produce one or both STxs.[31] The term enterohemorrhagic *E. coli* (EHEC) was proposed to denote those STEC strains that cause hemorrhagic colitis and, like infection caused by *S. dysenteriae* 1, the hemolytic uremic syndrome (HUS). Furthermore, EHEC or STEC strains like EPECs cause the attaching and effacing intestinal lesions on epithelial cells and some possess a 60-MDa plasmid that encodes for an hemolysin. STEC diarrhea is complicated by HUS in 2–10% of patients (especially children 1–5 years of age and among the elderly in nursing homes). Therapy of EHEC illness with certain antimicrobial agents (e.g., trimethoprim and fluoroquinolones) may predispose to HUS by encouraging the production of Stx, making therapeutic recommendations difficult. Not all antibacterial drugs appear to predispose STEC infection to development of HUS. STEC strains in the United States often belong to serotype O157:H7 or O157:H$^-$. EHEC belonging to O157 serotype characteristically do not ferment sorbitol, while >70% intestinal floral *E. coli* do. STEC belonging to other serotypes (non-O157) often ferment sorbitol like normal flora. STEC produce one or both Shiga toxin 1 (Stx 1) and Shiga toxin 2 (Stx 2). Stx 2 may be more pathogenic and more important in the production of HUS. To properly screen EHEC strains in cases of diarrhea, stool should be plated on MacConkey-sorbitol media and sorbitol-non-fermenting *E. coli* screened for O:157 LPS serotype. In addition, the stool sample should be tested directly for presence of Stx 1 and Stx 2 by EIA. There are reports from Europe of Shiga toxin-negative *E. coli* O157:H$^-$ causing HUS. EHEC infections are more common in the Northern and Northwestern United States. Two-thirds of symptomatic STEC infections are associated with the passage of bloody stools (dysentery), fever is characteristically absent with leukocytosis and abdominal tenderness commonly found. The highest incidence of disease is in the 5–9 years age group.

EAEC are increasingly being recognized as a cause of diarrhea in developing countries, especially persistent diarrhea in children and acute travelers' diarrhea. The organism may cause foodborne outbreaks in industrialized regions and appears to be an important cause of diarrhea in patients with AIDS worldwide. The organisms show an aggregative pattern of attachment ("stacked brick" appearance) in the HEp-2 cell assay with bacteria adhering to the cells and the glass substratum. The organisms often produce an inflammatory intestinal response as determined by presence of fecal lactoferrin and cytokines (interleukin 8 and IL-1b) during infection. The importance of mounting an IL-8 inflammatory response is seen in persons with a single-nucleotide polymorphism (SNP) in the promoter region of IL-8 gene who show an increased genetic susceptibility to EAEC diarrhea.[32] A variety of EAEC virulence factors have been described[20] explaining the propensity for virulent strains to produce diarrhea (see Pathogenesis and Immunity). Many EAEC do not possess these properties and may be avirulent. Foods in developing countries are commonly contaminated with the organism. A fluoroquinolone antibacterial drug and rifaximin were shown to be beneficial in shortening the diarrhea associated with EAEC infection in adult patients with AIDS and in travelers with diarrhea.[33,34] Human pathogenicity of at least one EAEC strain has been confirmed in adult volunteers.[35]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

The frequency of EPEC in stools of pediatric diarrhea is higher when hospital cases were compared to outpatients.[36] In the reports with the highest frequency of EPEC disease, a single strain usually could be identified in many of the cases, indicating a community-wide epidemic. Three geographically distinct areas were involved in one outbreak. In the three epidemic census tracts, attack rates were 73, 118 and 55 per 1,000 among nonwhite infants less than 1 year of age. Among index households, 50% had one or more asymptomatic pharyngeal carriers of the epidemic strain, while next-door neighbors and distant community household members harbored the strain in the pharynx in 33 and 0%, respectively. Intestinal carriage in the three different household groups was 18, 3 and 0%, respectively. Kessner et al.[37]

carried out a prospective examination of EPEC in a defined geographic area in Illinois and Indiana. An extensive community epidemic of diarrhea due to *E. coli* O111 during the summer, autumn and winter of 1960 and 1961 was documented. Age-specific attack rates for the 0- to 6-month-old age group ranged from 1,800 to 3,700 per 100,000. Carrier rates of asymptomatic contacts of index cases ranged from 5 to 10%. Pal et al.[38] described a community outbreak that occurred in India. The population morbidity rate was 0.16%, with age-specific attack rates for the 0- to 2-year-old age group of 0.98% and for the 6- to 11-month-old age group of 2.4%. During another community outbreak in Atlanta, EPEC strains were isolated from 12.7 per 1,000 persons at risk and from 125 per 1,000 infants less than 1 year of age. EPEC strains have been shown to be responsible for both epidemic and sporadic diarrhea.

While ETEC has been reported to cause a low frequency of diarrhea in the United States and Canada recently outbreaks of illness have been identified.[39] ETEC strains may produce epidemics of diarrhea. Such epidemics have been associated with the consumption of food or water contaminated with ETEC.[40] Also, outbreaks have been documented in hospital nurseries.[41] ETEC infection is more common in developing countries. The worldwide frequency of occurrence of bouts of ETEC diarrhea is estimated to be in the range of 650 million cases per year resulting in death in up to 380,000 children under 5 years of age. These organisms were the most frequently isolated pathogen among Mexican children with diarrhea.[42] Latin Americans possess high serum antibody titers to LT, while North Americans possess relatively low antibody titers.[24] The titer of LT antibody most probably reflects prior exposure to LT-producing *E. coli* and directly relates to resistance to infection. In a study carried out in rural Egypt, the incidence of ETEC infection was 1.5 episodes per child per year accounting for 66% of first episodes of diarrhea after birth.[43] ETEC strains were shown to be the most important cause of diarrhea among US travelers in Latin America,[44,45] being responsible for 40–60% of disease. As persons from the United States remain in endemic areas, they develop resistance to ETEC infection and diarrhea.

EIEC strains are unusual causes of endemic or epidemic diarrhea. Outbreaks associated with food contaminated with EIEC have been documented in the United States. EIEC has also been shown to be a cause of endemic diarrhea in Brazil[7] and among travelers to developing nations.[46]

STEC has been incriminated in both endemic and epidemic diarrhea in the United States, Canada, Europe and Japan. Outbreaks have occurred in nursing homes, day care centers, schools and the community; this class of pathogens also causes sporadic cases of colitis. Outbreaks of STEC have been associated with ingestion of inadequately cooked hamburgers, unpasteurized apple juice, wading pools, drinking water and produce. Recently exposure to animals in petting zoos has become a risk for infection. During 2005 and 2006, contaminated lettuce and spinach became an increasing risk for STEC disease transmission.[47]

EAEC are known to be important causes of diarrhea in certain high-risk groups. The strains cause between 10 and 20% of travelers' diarrhea[48] and an uncertain percentage of diarrhea in patients with advanced HIV infection.[49,50] A study of children with diarrhea carried out in Cincinnati between 1998 and 2000 revealed that EAEC strains were more commonly identified in inpatient children (5%) and children visiting emergency departments (10%) than in well children studied (1.4%).[51] Since routine diagnostic microbiology laboratories do not look for this organism we know very little about the frequency of occurrence in most populations.

5.2. Epidemic Behavior and Contagiousness

EPEC strains have a predilection for spread within hospitals, particularly in newborn nursery populations. Although the organism is spread within the community, the hospital probably plays a role in transmission to community members.[36]

Communicability of ETEC strains from person to person is not likely to occur[52] because of the high inoculum required for disease.[3] Epidemiologic and microbiologic investigations have implicated contaminated food as the most common vehicle for ETEC infection.[53–55] ETEC infections in areas of endemic infection tend to be clustered in warm, wet months, when multiplication of ETEC in food is most efficient.

Epidemic behavior and contagiousness of EIEC strains are unknown.

Very large point source outbreaks of STEC serotype O157:H7 have been recognized.[7,12] Other EHEC serotypes have been shown to cause endemic diarrhea and epidemic disease. Intrafamilial secondary transmission of O157:H7 has been documented to be ~10% during community outbreaks.[16]

EAEC is a high-inoculum[35] foodborne[55] disease. Person-to-person spread should occur only rarely if at all.

5.3. Geographic Distribution

EPEC diarrhea occurs with a worldwide distribution. The first large outbreaks were recognized in industrialized urban parts of the world. This may have been due to greater reporting with improved identification techniques. EPEC diarrhea has also been reported in studies of endemic diarrhea in developing countries. tEPEC is an uncommon

cause of diarrhea in industrialized countries most often found in children with diarrhea in these countries whereas in recent years atypical EPEC (aEPEC) strain identification in industrialized countries has increased.[1] Furthermore, tEPEC continues to be an important agent responsible for infantile diarrhea in developing countries. In recent years aEPEC has been associated with diarrhea documented in Mexico[56] and Brazil.[1]

Endemic ETEC diarrhea has been shown to be common only in the developing world. ETEC strains cause low level endemicity with occasional outbreaks in the United States. ETEC diarrhea has been reported to occur with high frequency in Latin America, Southern Asia and Africa.

EIEC strains have a worldwide distribution, but have been reported to be a frequent cause of diarrhea only in Brazil.[7]

STEC are predominately seen in the developed nations. The incidence of the organism in developing countries appears to be lower than in industrialized areas although strains have occasionally been isolated from patients with diarrhea in these regions.[56]

EAEC strains occur worldwide. As with many other bacterial enteropathogens, EAEC infection rates may be most common in developing regions where enteric disease is hyperendemic.[56–58] In Brazil, EAEC have been implicated in up to 68% of cases of persistent diarrhea in infants and the pathogen has been associated with deaths associated with diarrhea. EAEC have also been implicated as causes of sporadic diarrhea in Mexico, Chile, Bangladesh and Iran.

5.4. Temporal Distribution

EPEC diarrhea occurs year-round, with a summertime peak incidence. The time of year probably does not greatly influence disease rates.

ETEC diarrhea has been reported more commonly during warmer summer months which may relate to the season when most studies have been carried out. In two semitropical countries, Mexico and Morocco, ETEC infection appeared to be most common in rainy summer months.[59,60]

There is no information about the temporal distribution of EIEC, STEC or EAEC infections. As with other bacterial enteropathogens these infections are likely to be more common in the warmer summer months.

5.5. Age

One study examining the relationship of age to EPEC infection demonstrated that the median age at the time of hospitalization was 8 months and 2 weeks.[37] The distribution by age groups of hospitalized children was 86% between 0 and 18 months, 65% under 1 year of age and 9% in the neonatal period. In the community survey, the age distribution of those with positive stool cultures, regardless of symptoms, mirrored the age group hospitalized for diarrhea. Most published studies have confirmed that nearly all episodes of culturally confirmed EPEC diarrhea are seen in infants under 2 years of age, with more than half being in the group less than 6 months of age. In Houston, EPEC disease was documented to occur 20 times more frequently in infants below 6 months of age compared to children 1–2 years of age.[61] One study indicated that approximately 25% of infants were infected with an EPEC strain during the first year of life, and the prevalence of EPEC intestinal infection by a group of infants less than 1 year of age was 1%.[62]

In ETEC diarrhea, most cases of infection occur in children in areas where disease is endemic, as determined by the natural history of antibody to LT during the first 3 years of life plus the observation that adults living in these areas are immune to infection. In endemic areas, the highest attack rate of ETEC infection occurs in the age group 6–36 months of age, with decreasing rates with increasing age.[63] Although ETEC infection occurs most frequently in infants, immunological naïve adults are susceptible. ETEC is the predominant etiologic agent causing travelers' diarrhea among adults from the developed world visiting areas where ETEC is endemic.[64] Studies suggest that 20–60% of such travelers experience diarrhea with 20–40% of cases being due to strains of ETEC.

No information is available about the relationship of age to EIEC diarrhea. Like all foodborne bacterial diarrheas, it is likely to be most common in young children and in any age group consuming food contaminated by the organism.

While STEC can infect any age group, STEC diarrhea is most common in children. Hemolytic uremic syndrome following STEC infection characteristically occurs in children although this complication also occurs in the elderly.

Association of age with EAEC diarrhea has not been studied. Clearly all age groups are susceptible to EAEC diarrhea.

5.6. Sex

As with many other infections, males of all ages show a higher infection rate due to EPEC strains. An overall male–female ratio of 4:1 has been reported.[36] Insufficient information is available to determine whether a sex relationship exists for the other diarrheal diseases due to *E. coli*.

5.7. Race

The published studies show a correlation of EPEC infection with exposure to hospitals and crowded living conditions, which probably explains the greater occurrence

of EPEC infections in nonwhite children in the United States.[36]

Insufficient data are available to determine whether there are racial influences on rates of ETEC, EIEC, STEC and EAEC diarrhea; however, race is not likely to be a risk factor for these enteric infectious diseases.

5.8. Occupation

EPEC is a disease of infants. Those persons taken into tropical climates by their employment might be expected to be at risk for ETEC disease. Occupational factors relating to exposures to EIEC, STEC and EAEC are unknown.

5.9. Occurrence in Different Settings

EPEC strains produce diarrhea primarily in hospital populations, particularly in nurseries housing newborn infants. Hospitalized infants and children attending day care centers represent the major reservoirs for community spread.

ETEC diarrhea is a particular problem among groups traveling from low-risk areas (e.g., United States, Northwestern Europe, Canada, Australia, New Zealand and Japan) to high-risk areas (Latin America, Southern Asia and Africa). It is also a problem among infants and children in these high-risk areas. Hospital outbreaks of ETEC diarrhea have been reported.[41]

EIEC organisms have been reported as a rare cause of foodborne outbreaks in the United States.[8–10] These organisms are uncommon causes of diarrhea in most parts of the world. In Brazil there appears to be an unexplained high prevalence of EIEC-associated diarrhea.[7]

STEC diarrhea and dysentery have been shown to occur primarily in developed nations.[11]

EAEC strains have been identified in travelers with diarrhea to a variety of countries,[48] in children from developing countries,[42,56,57] in AIDS-associated diarrhea in the United States and Africa and in infants in developing countries.

5.10. Socioeconomic Factors

Socioeconomic factors are important in EPEC diarrhea primarily as they influence crowding, sanitation, breastfeeding practice and exposure to hospitals. There is no known relationship between socioeconomic factors and other diarrheal diseases due to *E. coli*, although it can be assumed that reduced socioeconomic level would relate to increased rates of diarrhea due to each of the diarrheagenic *E. coli*.

6. Mechanisms, Routes of Transmission and Reservoirs

Within hospital environments, EPEC organisms are transmitted by direct contact, spread by dust and by fomites. During one outbreak of diarrhea due to an EPEC strain, nursing personnel and family members were shown to be asymptomatically infected with the epidemic strain,[65] suggesting a possible role in the transmission of the agent. In the same study, recovery of an EPEC from stools of mothers just before delivery was documented in 13%, with subsequent isolation of *E. coli* with the same serotype from 40% of the newborn infants of the culture-positive mothers. Asymptomatic adult carriers of EPEC strains undoubtedly play a role in disease transmission to infants and young children. During one community epidemic, the infecting strain was commonly isolated from pharyngeal cultures, and respiratory symptoms were noted.[65] As is the case with other enteric pathogens, an association of respiratory symptoms and EPEC diarrhea has been reported. It is possible that a respiratory route of transmission may play a role in disease transmission with this and other enteropathogens. EPEC strains as with other enteric pathogens are spread by the fecal–oral route of transmission. Person-to-person contact spread has been suggested by the epidemiological patterns of EPEC infection. Typical EPEC serotypes have not been found in animals, suggesting that humans are the only living reservoir for these organisms.[1] In contrast, most atypical EPEC serotypes have been isolated from a variety of animal species and likely both humans and animals may serve as reservoirs for aEPEC.[1]

ETEC strains are characteristically spread through ingestion of contaminated food.[53–55,66] In our studies in Mexico, when students ate at public eating establishments, they were more likely to develop ETEC diarrhea than if they consumed meals prepared by themselves in their own apartments. Food sampled from public eating facilities was commonly found to be contaminated with ETEC.[53–55,66] Due to the large inoculum necessary to produce diarrhea in healthy adults ($>10^8$ viable cells),[3] it is likely that food and less likely water are the important vehicles of disease transmission. Only humans have been described to be reservoirs for human ETEC.

The important transmission kinetics of EIEC is unknown, largely because of the rarity of infection by these strains. The major documented outbreaks have been associated with consumption of food contaminated with these organisms.

It is known that STEC strains can commonly be identified on the surface of beef secondary to enteric infection of cattle by the organisms.[11,16] Lake and wading pools may be a source of infection. Other foods and beverages including

drinking water may be the source of infection.[67] A variety of animals including sheep, pigs, goats, cats and dogs can harbor STEC strains in their intestine, but cattle are considered to be the most important animal species in terms of human infection.

The source of EAEC strains in developing regions is likely food.[55]

7. Pathogenesis and Immunity

Several histopathologic studies have shown that there is a characteristic lesion associated with EPEC infection.[27] The EPEC are seen by electron microscopy as microcolonies that adhere to epithelial cells with destruction of the brush border, but with no evidence of invasion. Pedestal formation has also been noted. This lesion can be duplicated in an animal model[68] and there is a tissue culture model of infection (HEp-2 cell assay).[28] This adherence property is encoded for by genes carried on a plasmid known as the EPEC adherence factor (EAF). The EAF plasmid encodes a type IV pilus called the bundle-forming pilus (BFP) which mediates adherence. Production of toxins has been described for EPEC strains and the intestinal lesion suggests damage from unrecognized toxins. Although EPEC strains appear to produce illness in adults, the disease is primarily a disease of newborns and young infants. The age distribution suggests that protective immunity develops. The nature and extent of immunity are unknown.

Prior to the development of illness, ETEC strains must colonize the toxin-sensitive upper intestine before elaborating secretory enterotoxins. Specific fimbrial antigens on the surface of ETEC strains have been shown to mediate adherence of the *E. coli* to the mucosa of the small bowel. Table 2 lists the recognized colonization factor antigens of ETEC according to natural hosts. In the small intestine of piglets, the K88 antigen appears to bind to a receptor on the villous epithelial cells.[69] Attachments of ETEC to the upper gut depend not only on the fimbriae of the bacteria but also on the presence of specific receptors for the fimbriae.[70] Some strains of pigs genetically lack the receptor for K88, making them immune to disease caused by K88-bearing organisms. Antigenically different fimbriae, K88, K99, 987-type and type 1 (common) pili, adhere equally to porcine intestinal epithelium.[71] These ligands probably bind to different receptors in view of a lack of competitive inhibition of bacterial adherence. ETEC strains infecting humans usually possess one of several recognized colonization factor antigens known as CFA/I, II, III and IV and putative colonization factors (e.g., PCFO159 and PCFO166).[72]

Table 2. Adherence Fimbriae of Enterotoxigenic *E. coli* (ETEC)

Natural Host	Colonization Factor Antigen (CFA)	Coli Surface (CS) Adhesin
Piglet		K88
Calf, lamb		K99
Piglet		987-type
Human	CFA I	
	CFA II	CS 1, CS2, CS3
	CFA III	CS 8
	CFA IV	CS4, CS5, CS6
		Miscellaneous surface adhesins: CS7, CS12 (PCFO159), CS14 (PCFO166), CS17

Natural immunity to ETEC infection occurs when persons remain at high risk for infection. In a study of students attending school in Mexico, the attack rate was inversely related to the length of time the student had lived in the endemic area.[44,73] Newly arrived students from the United States suffered from diarrhea at a rate of 40% per month, compared to a rate of 20% for the same time period for those students from the United States who had been in Mexico for approximately a year or longer. Newly arrived students from Venezuela did not show an increase in illness, indicating immunity from prior exposure in Venezuela to agents prevalent in Mexico.[73] A decreased frequency and lowered severity of illness among Latin American students correlated with an apparent resistance to ETEC infection. An inverse relationship was found between the geometric mean antibody titer (GMT) to LT and the occurrence of diarrhea associated with LT-producing *E. coli*.[24] The lack of exposure to ETEC in the United States appears to explain the remarkable susceptibility to infection among persons from the United States traveling in developing countries. Immunity to ETEC infection does not appear to be strictly antitoxic in origin, since Latin American students exposed to ETEC strains rarely excrete the organism in stools asymptomatically, in contrast to the common finding of asymptomatic fecal shedding of strains in newly arrived US students.[44,74] These data suggest that antibacterial immunity is operative because virulent strains are excluded from gut colonization. This could represent intestinal antibody to adhesion of fimbriae and/or somatic antigens or other factors.

The pathogenic mechanisms of EIEC are the same as those of *Shigella*. These organisms invade the colonic mucosa and spread laterally in the epithelium, causing ulcer formation and clinical dysentery. It can be assumed that immunity to EIEC is similar to that seen in shigellosis where immunity is known to be serotype specific.

E. coli O157:H7 strains have been shown to elaborate important quantities of Shiga-like toxin that is thought to be responsible for the tissue damage seen in this infection. Serum antibodies to Shiga-like toxin and to lipopolysaccharide of the O157 antigen have been demonstrated in patients with STEC diarrhea.[25]

EAEC possess a range of virulence properties involved in three stages of disease production: adherence to intestinal mucosa; increased production of mucus; and production of an intestinal inflammatory response.[75,76] Adherence is mediated by plasmid-controlled fimbriae and other adhesins that have been partially characterized.[76,77] Strains producing infection and disease are characterized by their heterogeneity.[20,77,78] Aggregative attachment is important in EAEC strains and a wide number of virulence factors have been found with EAEC strains isolated from human subjects. A number of putative and known virulence have been described. Mucosal adherence appears to be related to production of a variety of adhesins including fimbrial structures referred to as aggregative adherence fimbriae (AAF) important to the aggregative adherence capacity of the organism and nonfimbrial adhesins. AAF secretion apparatus transports to the mucosal surface where dispersin protein appears to be important in local dispersal of the bacteria. Many EAEC strains harbor chromosomal factors including mucinase (Pic) and *Shigella* enterotoxin 1 (ShET1) and enteroaggregative heat-stable enterotoxin 1 (EAST-1) and the plasmid autotransporter cytotoxin, Pet. EAEC induce an inflammatory response with release of IL-8 from epithelial cells possibly mediated by presence of bacterial flagellin. Adherence is encoded in some EAEC strains by the adherence plasmid pAA including aggregative adherence fimbriae AAF/I and AAF/II. Nataro has described a transcriptional activator which controls virulence factor expression in a large number of pathogenic strains of EAEC.[20] The fact that no one virulence factor is found in all pathogenic strains of EAEC and that EAEC strains with definable virulence properties are commonly isolated from persons without symptoms complicates the field.

An interesting line of research has dealt with genetic differences in susceptibility to enteric infection. Based on the finding of high levels of IL-8 in stool samples of persons with bacterial diarrhea our group has looked at IL-8 genes and susceptibility to infection and diarrhea by these bacterial pathogens. Presence of polymorphism in the promoter region of the IL-8 gene was shown to be associated with increased susceptibility to EAEC diarrhea.[32]

8. Patterns of Host Response

In EPEC infection, unapparent infection and transient colonization are common, especially among older children and adults. In one study, 15–20 per 1,000 normal children between birth and 5 years of age harbored an EPEC strain in their stools.[62] In a 1-year longitudinal study of 40 households, transient excretion, infection defined as excretion longer than 4 weeks, and clinical illness were most common in young children.[79] The rate of excretion of EPEC in this study was approximately 11% in children under 1 year of age, while the rate in adults was 5%. Overt illness was seen in 25% of children under 1 year of age who had an EPEC detected in their stools. Illness is uncommon in older children and adults, even though they often carry the organism.

Asymptomatic ETEC infection commonly occurs in persons exposed to these strains in endemic areas.[44,74] It appears that highly susceptible individuals most commonly develop overt disease when exposed to ETEC strains, in contrast to those partially immune, in whom excretion without illness characteristically occurs, and to those with natural immunity through prior exposure, in whom neither disease nor excretion can be documented.

Asymptomatic excretion of EIEC was shown to be common in experimentally challenge in volunteers and seroconversion occurred without overt illness.[3]

STEC are low-dose pathogens causing illness in a high percentage of exposed persons. For unclear reasons, STEC diarrhea is most common in industrialized regions where children are exposed to the organism in food, water or from person-to-person spread.

All patient groups appear susceptible to EAEC strains. In highly endemic areas, such as Mexico, symptomatic disease occurs early after exposure followed by low rates of illness in the face of high infection rates.[80] Recently a study documented that EAEC diarrhea occurred commonly in US children.[51] Our group found that the presence of *aggR*-dependent virulence factor was associated with the presence of enteric symptoms. These data support heterogeneity of virulence among strains defined as EAEC by adherence assay.[78] As already presented, persons positive for a single-nucleotide polymorphism at the IL-8 promoter region are associated with increased rates of EAEC diarrhea among US students in Mexico.[32] These data suggest that both host and strain factors may contribute to susceptibility.

8.1. Clinical Features

While there is marked variation in the duration and severity of EPEC illness, the most common findings are fever (60%), diarrhea (>90%), respiratory symptoms (50%), abdominal distension suggesting paralytic ileus (10%) and rarely convulsions (<1%).[37] Dehydration may be seen in one-third of cases, especially in infants under 1 year of age, and hypernatremia and metabolic acidosis occur. Dysentery

has been reported in some cases of EPEC diarrhea. Illness usually lasts about 1 week, yet protracted illness commonly occurs and the illness may result in fatalities.[14,36] In fatal cases and in certain outbreaks of more virulent disease, a picture of septicemia and widespread intestinal inflammation and necrosis are found. A more severe disease with a higher percentage of fatalities has been seen in infection with the O111 serogroup of *E. coli*.[14,79] Mortality is higher for ill infants who are less than 1 month of age.[37]

In ETEC diarrhea, the disease resembles mild cholera in most cases, where the most common finding is watery diarrhea. In contradistinction to cholera, abdominal pain and cramps are common in *E. coli* diarrhea and may be severe and low-grade fever may be seen. An inflammatory nature of the intestinal lesion in ETEC diarrhea is suggested by the finding of fecal cytokines in naturally occurring illness.[81] In our experience in Mexico, the average untreated illness in adults consists of passage of 8–12 unformed stools over a 4- to 5-day period.[73] In volunteers with experimentally induced illness, the clinical syndrome in the most severe cases consisted of passage of 5–10 watery stools per day, lasting as long as 19 days. We have seen a patient in Houston with ETEC infection present with a cholera-like illness requiring more than 30 l of intravenous fluids to maintain hydration. Of significance was that this person had had a previous gastric resection for peptic ulcer disease. Fatalities are unusual with ETEC disease and probably occur only in the very young, the elderly and those with marginal cardiovascular reserves. The illness associated with ST-only (strains that produce only ST) *E. coli* appears to be quite similar to that produced by ST/LT strains.[41]

EIEC strains produce an illness indistinguishable from shigellosis (bacillary dysentery). Fever, abdominal pain and passage of bloody mucoid stools are frequently seen.[3]It seems that EIEC severity can be increased in malnourished children coinfected with other pathogens.[82]As in shigellosis, the disease is usually self-limiting, and fatalities are probably unusual.

Hemorrhagic colitis secondary to STEC infection is a distinctive clinical syndrome.[11] It normally starts as watery diarrhea with abdominal cramps and progresses in a majority of cases to grossly bloody diarrhea with little or no fever. The average duration of symptoms is around 8 days. There does appear, however, to be a spectrum of clinical disease with some patients having only watery diarrhea with no blood in the stool or fever. STEC infection has also been associated with hemolytic uremic syndrome as a common sequela to infection with these organisms.[11]

EAEC is a non-invasive but inflammatory pathogen characteristically causing watery diarrhea without high fever or dysentery.

8.2. Diagnosis

The features of *E. coli* infections relevant to diagnosis are summarized in Table 3. EPEC diarrhea should be considered in any outbreak that develops in a hospital nursery or on a pediatric hospital ward. In this setting, serotyping or virulence testing of *E. coli* isolated from affected infants may identify the outbreak strain. Because of a variable clinical syndrome depending on strain virulence and host response, any diarrheal illness in a hospitalized pediatric population should be considered as potentially EPEC in origin. Serotyping or virulence testing of *E. coli* from children with sporadic community-acquired diarrhea does not justify the cost. More studies need to be conducted to determine the importance of the HEp-2 cell adherence assay and the EAF probe in detecting EPEC strains in endemic diarrhea.

ETEC intestinal infection should be considered in any traveler from a low-risk area such as the United States, Canada, Northwestern Europe, Australia, New Zealand or Japan who develops diarrhea without high fever while traveling in Latin America, Southern Asia or Africa. Unfortunately, the routine diagnostic laboratory is presently unable to assay for LT and ST, which are available only in research and reference laboratories. ELISAs, DNA probes and PCR methods are now being developed for use in the routine diagnostic laboratory.

EIEC infection should be suspected in a patient with bacillary dysentery (passage of small volume bloody mucoid stools with prevalent fecal leukocytes on microscopic examination) in whom stool cultures are negative for *Shigella* and *Campylobacter*. The routine diagnostic laboratory can make a presumptive identification of EIEC by the biochemical reactions of these organisms. EIEC strains, unlike most *E. coli* strains, are lysine decarboxylate negative and are nonmotile. Presumptively identified strains must then be confirmed in a reference laboratory by DNA probe or the Sereny (guinea pig eye) test.

STEC diarrhea should be suspected in outbreaks of bloody diarrhea in which the affected patients have little or no fever. The infecting strains can be definitively identified only by demonstrating Sxt 1 and Sxt 2 production or possession of genes encoding for Shiga toxins. EHEC strains can belong to many *E. coli* serotypes (see Table 1), but O157:H7 and O26:H11 are the most common. The clinical laboratory can screen for O157 strains by looking for *E. coli* that fail to ferment sorbitol, confirmed to be LPS type O157 and whose motility can be blocked by antisera to H7.

Multiplex PCR for identification of EPEC (*eae* A, *bfp*), ETEC (ST, LT), EIEC (*ial*) and STEC (stx1, stx2, *eae* A) has shown to be useful for large epidemiological studies.[56,57]

EAEC identification will remain a diagnosed condition only by research laboratories until efficient laboratory reagents are available.

Table 3. Features of Diarrheagenic *E. coli* Infections

	EPEC	ETEC	EIEC	STEC	EAEC
Laboratory diagnosis	Serotyping, DNA hybridization, PCR	ST and LT detection by ELISA, DNA hybridization or PCR	Sereny test, DNA hybridization, PCR	Serotyping or Shiga toxin production by EIA	HEp-2 cell adherence or PCR
Prevalence	Occasional cause of hospitalized diarrhea among infants	Occasional occurrence in United States, causes travelers' diarrhea in 40–60%	Rare	Increasing outbreaks of foodborne dysentery, occasional hemolytic uremic syndrome	Causes persistent pediatric diarrhea in developing regions, occasional cases of acute diarrhea in industrialized areas and AIDS-associated diarrhea
Outbreaks	Nurseries	Travelers, infants, foodborne	Foodborne	Foodborne, less commonly waterborne outbreaks	Foodborne diarrhea outbreaks in developing regions
Geographic	Worldwide, more common in developing countries	Developing nations, occasionally in United States and developed regions	Worldwide, more common in Brazil and in Eastern Europe	Developed countries or countries of the southern hemisphere (Argentina, Chile, South Africa)	Worldwide, more common in developing countries
Temporal	Summer peak	Year-round, summer peak	Unknown	Summer peak	Unknown
Age	<2 years, more common ≤6months	Travelers, children	Unknown	All ages, most common in children	Most common in infants and children

9. Control and Prevention

9.1. General Concepts

Since *E. coli* diarrhea is not a reportable condition, we do not have adequate information about the epidemiology and optimal control and intervention procedures. It is clear that during EPEC outbreaks, standard public health and infection control procedures are important in terminating transmission. Isolation of cases, cohorting by area and nursing personnel, institution of strict hand washing and prevention of common exposure to equipment, bedding or solutions are all indicated.

In ETEC diarrhea of travelers, careful selection of food by individuals and institutions and enforcement of food hygiene in public eating establishments by departments of public health are the major areas for disease control. In high-risk areas, ingestion of steaming foods and beverages, carbonated bottled drinks and citrus fruits and avoidance of moist foods maintained at room temperature (e.g., leafy green vegetables, cold meats, desserts, fresh cheese and milk) are advisable when traveling in parts of the world where food sanitation is substandard. While consumption of tap water in the developing world is not advisable, using this water for rinsing the mouth and toothbrush is generally safe.

In EIEC diarrhea, it is not known whether person-to-person spread occurs but considering the relatively large inoculum responsible for disease, it is likely that food or less commonly water represent the important vehicles of transmission. No specific information on the control of EIEC is available.

STEC infection is best prevented by avoidance of undercooked beef, especially hamburger, monitoring of other foods including produce and adequate chlorination of water to which persons are exposed.

There are no known principles of prevention of EAEC diarrhea other than avoiding consumption of contaminated food which may be possible for travelers by eating only steaming hot foods.

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis

The most important factor in treatment of any diarrheal disease is the maintenance of fluid and electrolyte balance by administering oral rehydration fluids. The value of antimicrobial agents in the control of hospital outbreaks of EPEC diarrhea has not been established.

Antibacterial drugs will significantly shorten the duration of ETEC diarrhea and EAEC diarrhea as studied in travelers during short-term stay in endemic areas. The antimicrobial drugs of value include rifaximin,[83] the fluoroquinolones[84] and azithromycin.[84] In studies of ETEC and EAEC diarrhea, a variety of antimicrobial agents have been used with success in preventing illness among travelers from low-risk to high-risk areas. The most active drugs are the fluoroquinolones and non-absorbed (<0.4%) rifaximin. Of these only rifaximin can be recommended for general use as a chemoprophylactic agent, based on efficacy, safety and minimal concern about development of resistance for a non-absorbed drug.[85] Rifaximin may be recommended for diarrhea prevention among travelers to high-risk areas for those on a tight schedule, when illness has occurred during past trips (suggesting increased susceptibility) and when it is requested by the future traveler.[86] A fourth group in which to consider use of rifaximin prophylaxis is the group with immunosuppression or where the underlying illness could predispose to complications if diarrhea developed. It is likely that rifaximin would also prevent EIEC diarrhea in high-risk settings.

In STEC infection, antibiotics have not been clearly demonstrated to be of value. Certain antibacterial drugs may lead to increased production of Shiga toxin and potentially encourage the development of the hemolytic uremic syndrome (HUS).

9.3. Immunization

No immunizing agents are available for EPEC, EIEC or EHEC infections.

Efforts are currently under way to develop a vaccine against ETEC. The impetus for this is two-fold. First, ETEC strains are major and predictable causes of diarrhea in international travelers, the military and state department employees traveling to developing regions and among children living in these areas. Second, ETEC immunity occurs naturally. In an ETEC-endemic area a longitudinal study of protection from natural ETEC infections in children revealed that ETEC infections produced 47% protection against new infections with strains of ETEC showing the same toxin CFA profile.[87] The authors provided evidence that for breastfed children living in endemic areas other antigens are substantially more important than CFAs for induction of ETEC-protective immunity. An orally administered cholera vaccine made of killed ETEC whole cells (expressing CFAI, II and IV) and the binding subunit of cholera toxin is currently available in some areas of the world for prevention of ETEC diarrhea.[88] In the published studies, short-term protective efficacy was particularly impressive for LT-ETEC diarrhea causing life-threatening dehydration; however, protection against ST-ETEC strains was not evident. Heat-labile enterotoxin (LT) applied as a transcutaneous antigen has been shown to be immunogenic.[89] The LT patch vaccine effectively prevented moderate to severe travellers' diarrhoea in a phase II clinical trial.[90] The approach is currently being pursued for commercial development as an immunizing agent for travelers and other high-risk populations.

10. Unresolved Problems

In tEPEC disease, the most important issue to be resolved is the current importance of this group of organisms which are rarely studied today. Regarding aEPEC, studies are needed to determine if these organisms are human pathogens and if so how often they produce enteric disease and in which populations.

In ETEC diarrhea, the most pressing problems center around the development of assays to efficiently identify ST, LT and CFAs in the routine diagnostic laboratory and successful development of protective immunoprophylactic agents.

The prevalence, epidemiology and improved diagnosis of EIEC diarrhea are needed to be clarified further.

Much needs to be learned concerning the prevalence, epidemiology, pathogenic mechanisms, detection and immune response to Shiga toxin-producing *E. coli*. Important questions relate to the exact relationship of EHEC infection to the development of HUS and the role of antimicrobial agents in disease pathogenesis and therapy.

Diarrheagenic *E. coli* comprise a diverse group of strains that should be classified by the pathogenic mechanisms they possess. Improved definitions of diarrheagenic *E. coli* should be given priority.

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12. Suggested Reading

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CHAPTER 16

Gonococcal Infections

Jonathan M. Zenilman and J. McLeod Griffiss

1. Introduction

Despite currently effective means of diagnosis and treatment, gonorrhea remains a major public health problem throughout the world. In 2005, 339,593 cases of gonorrhea were reported to the Centers for Disease Control by local and state health departments, [1] representing a nationwide population-based incidence case rate of 115/100,000. From 1975 through 1997, the national gonorrhea rate declined 74.3% following implementation of the national gonorrhea control program in the mid-1970s. Gonorrhea rates subsequently appeared to plateau for several years; however, the gonorrhea rate has decreased 11.8% from 2000 to 2004. An additional one to two cases are thought to occur for every reported case.[2] The highest rates are seen in adolescent women (aged 15–19 years) and young adult men (aged 20–24 years) representing, in part, sexual behavior patterns. *Neisseria gonorrhoeae* is a major cause of pelvic inflammatory disease,[3,4] which in turn causes tubal factor infertility and ectopic pregnancy. Gonococcal infection also facilitates human immunodeficiency virus (HIV) transmission.

The epidemiology of gonorrhea is determined by the complex interaction of human sexual behavior, social and health-related factors, and the biological characteristics of the organism. Prevention and control of gonococcal infection depend on individual and community education, high standards of diagnosis and treatment, appropriately targeted screening, counseling, and contact tracing.

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2. Historical Background

Allusions to gonococcal infection may be found in ancient Assyrian and Chinese writing, as well as in the Book of Leviticus (15:2). Relatively clear clinical descriptions were made by Hippocrates and Galen, who mistook the inflammatory discharge (leukorrhea) for semen—hence, "gonorrhea" or a "flowing of seeds". Between the 14th and 17th centuries, "gonorrhea" was considered by many to be just another manifestation of syphilis. However, Phillipe Ricord showed that it was a unique, transmissible disease by performing a series of inoculation studies in the 1830s, and Albert L.S. Neisser reported from Breslau (Wroclaw) in 1879 that he had observed "micrococcenform" in secretions from patients with gonorrhea with the use of stains and a Zeiss microscope outfitted with a newly developed condenser and oil-immersion system.[5] The organism was cultured by Bumm in 1885.

Early treatments of gonorrhea consisted of either ineffective oral preparations or potentially dangerous and caustic intraurethral injections. Urethral strictures and secondary infections were common, both as complications of the disease and/or of therapy. The first advance in treatment was reported in 1881 by Carl Credé, in Leipzig, who noted that ophthalmia neonatorum occurred only in infants born of mothers with vaginal discharge; he reduced the rate of this ophthalmic complication of gonococcal infection of the birth canal from $>13\%$ to 0 by instilling silver nitrate drops in the eyes of newborns at the time of birth.[6] Topical treatment at birth remains a mainstay in preventing this infection. The second major advance was the discovery of penicillin in the 1940s, which provided the first hope that gonorrhea could be eradicated. Despite some initial success, the 1960s saw a precipitous 12-year rise in gonorrhea incidence worldwide.[7] At the same time, relative resistance to penicillin gradually increased. In 1976, the introduction of plasmid-mediated

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β-lactamase-producing strains foreshadowed an end to penicillin's role as the treatment of choice.[8] Widespread resistance to penicillins and tetracyclines since the 1980s has led to a reliance on newer agents, such as third-generation cephalosporins and quinolones.

The last two decades have witnessed a tremendous expansion in our understanding of the gonococcus and the human response to infection. However, despite this expanding understanding, how best to formulate a successful vaccine remains unclear. At the beginning of the 21st century, the incidence of gonorrhea is more likely to be changed by time-tested public health measures than by advances in molecular biology.

3. Methodology

3.1. Sources of Mortality Data

Fatalities from acute gonococcal infection are rare events, usually resulting from endocarditis or meningitis complicating disseminated gonococcal infection (DGI).[9] Data on mortality from the sequelae of gonococcal infection, such as pelvic inflammatory disease (PID) and ectopic pregnancy, are unreliable because the conditions listed on death certificates are rarely specifically linked to the infection. For example, one study estimated the mortality from PID (all causes) to be 0.29 deaths per 100,000 women aged 15–44 years.[10] However, because the underlying microbial etiology is not listed, determining how many of these were related to gonococcal PID is impossible.

3.2. Sources of Morbidity Data

Gonorrhea is a reportable disease in all 50 states. Reporting criteria are based on standardized surveillance definitions that may be clinically or laboratory based.[11] Physicians, laboratories, and institutions have been required to report either positive diagnostic test results or the diagnosis, name, address, demographic data, and identity of the reporter to local or state health departments, depending on the jurisdiction. Despite these requirements, there is marked underreporting; an estimated 50–70% of cases are not brought to the attention of local public health authorities.

Data on all reportable infectious diseases, including sexually transmitted diseases (STDs), are reported by the states to the federal Centers for Disease Control and Prevention (CDC). The weekly incidence of gonorrhea is reviewed in the CDC publication *Morbidity and Mortality Weekly Report*. National statistical summaries of STD data are published annually.[1] In addition, antibiotic resistance surveillance is conducted through a variety of mechanisms. The United States and other countries (e.g., Canada and Australia) monitor antibiotic resistance through gonococcal isolate surveillance projects which assess periodically collected samples of gonococcal isolates.[12,13]

In the US military, gonorrhea and other STD reporting is a function of the Prevention Medicine Units (PMU) or infectious disease units for each of the armed services. Military bases located in the United States usually coordinate reporting and disease intervention activities with local county or state health departments. Bases located overseas report STD morbidity to the appropriate Preventive Medicine Command through the PMUs.[14]

Outside the United States, reporting requirements vary. Most countries do not have national-based surveillance for gonorrhea. The areas with the most complete reporting of gonococcal disease are Great Britain and the Scandinavian countries.[15,16] Data from other countries may rely on reports from STD clinics alone. For a case to be included in the national morbidity statistics, it first must be diagnosed, then reported, and finally correctly tabulated. Misdiagnosis, underdiagnosis, and underreporting probably occur frequently. The extent of these errors, or reporting bias, is partially determined and corrected by special surveys. Interpretation of disease incidence data must be carefully done, taking into account possible confounding factors, such as reporting bias, demographic trends, underreporting, and sexual behavior.

3.3. Surveys

Traditional morbidity reporting schemes are often biased because of substantial private sector underreporting. The development of new, easily fielded nucleic acid diagnostic testing has greatly facilitated and expanded the capacity to perform population-based field surveys to assess gonorrhea prevalence. For example, a large nationally representative survey of adolescent high school students (Add Health) found that a prevalence estimate of gonococcal infection was 0.4% (or 4 per 1,000 persons) among young adults aged 18–26 years.[17] The highest prevalence was in the South at 0.6%, reflecting a historical pattern that has been observed for >50 years. The prevalence among men and women was the same (0.4%). The prevalence of gonococcal infection was substantially higher among African-American men (2.4%) and women (1.9%), as compared to whites (0.1% in both men and women). In a population-based survey in Baltimore, the estimated prevalence was considerably higher than most other population-based estimates.[18] Overall, the prevalence was 5.3% among adults aged 18–35 years.

Among African-American women, the prevalence was 9.3% and among African-American men, the prevalence was 5.3%. The prevalence was 1.3% in both men and women for whites.

Screening has been especially useful in identifying asymptomatic infection in specific populations at high risk for gonococcal infection. Large-scale screening of US military recruits at induction found prevalence rates of 0.6% in men[19] and 5% in women.[20] Persons incarcerated at short-term detention facilities are at high risk for STDs; in Baltimore, the prevalence for gonorrhea is 3.4%.[21] Studies in emergency departments in urban areas such as Baltimore have found asymptomatic gonorrhea prevalence rates as high as 5.3%.[22]

Screening programs and surveys have served multiple purposes in guiding the emphasis of national and state control programs. For example, the National Gonorrhea Screening Program, which started in 1972, provides federal support to the states for gonococcal diagnostic testing and has been instrumental in identifying large numbers of asymptomatically infected women.

Demographic surveys have indicated that persons infected with gonorrhea are geographically concentrated and that targeting intervention efforts in these areas may be productive. An interesting use of reported case data for gonorrhea is the spatial representation of cases using geographic information systems (GIS). To assess the distribution of cases in a city or county, the addresses of gonorrhea cases, their partners, or the venues where partners were met are "geocoded", or placed on a map using coordinates. The cases are then aggregated to a larger geographical unit, such as the zip code or census block. The distribution of disease can then be represented as case counts, case density (number of cases over the area), or incidence rate (number of cases over the number of persons living in the area). Application of these techniques in many urban areas such as Baltimore, Seattle, and Colorado has provided[23–25] useful information regarding the core areas of gonorrhea transmission. Such information can be used to target interventions to specific areas with high incidence of disease (see Section 9.2).

Microbiological surveys, such as the national Gonococcal Isolate Surveillance Project,[12] which started in 1986, have been useful in determining the prevalence of gonococcal strains with antimicrobial resistance. These data, in turn, may be useful in developing recommendations for therapy.

3.4. Laboratory Diagnosis

3.4.1. Isolation of the Organism. Culture of clinical specimens for the organism requires supplemented agar media, such as "chocolate agar", which contains hemolyzed sheep's blood. Since genital sites are not sterile, in order to prevent overgrowth of contaminating microorganisms, selective media, such as modified Thayer–Martin or Martin–Lewis media, are used.[26] These media contain antibiotics (e.g., vancomycin, colistin, and nystatin) that suppress fungi, gram-negative rods, and gram-positive bacteria. However, a very small proportion of gonococci are hypersusceptible to vancomycin and may fail to grow on selective media.

Presumptive identification may be made on the basis of growth on selected media, characteristic colonial and microbial morphology, and a positive oxidase test. The gonococcus lacks the enzymes maltase, sucrase, and lactase that split glucose from complex disaccharides; it can metabolize glucose, but not maltose, sucrose, or lactose, and this pattern of use distinguishes it from other *Neisseria*. Final identification may be confirmed by immunologic or molecular biology techniques. A variety of immunologic confirmatory tests, such as direct fluorescent polyclonal or monoclonal antibody stains, are commercially available.

Specificity of culture without confirmation varies by anatomical site and the concomitant presence of commensal organisms that could produce false-positive results. Specificity of presumptive laboratory diagnosis of urethral cultures (i.e., identification without confirmation by sugar use or monoclonal antibodies) in men is over 95%. For cervical specimens in women, specificity is around 85%.[27] In pharyngeal, rectal, and ocular infections, the specificity is lower and all isolates from these and other unusual sites should be confirmed. *All* isolates from children and others in whom sexual abuse or assault is a consideration should be confirmed and saved.

In practice, cultures should be obtained from the anatomical sites corresponding to the patient's sexual exposures by history. Pharyngeal and rectal cultures should be performed if indicated. Because up to 30% of women have rectal coinfection with cervical gonorrhea (irrespective of history of rectal intercourse), routine rectal culture is recommended for all women.

Compared to nucleic acid amplification tests, estimates of culture sensitivity are 90–95% for urethral cultures in symptomatic men and 50–80% for asymptomatic male urethral or cervical infection.[28,11] Rectal cultures probably have a sensitivity similar to cervical cultures. Therefore, either site may be the sole site where infection can be detected. Pharyngeal culture sensitivity is probably somewhat less. In all cases, specificity should approach 100% if confirmatory procedures are used.

Laboratory diagnosis of gonococcal infection from normally sterile sites, such as blood, synovial fluid, cerebrospinal fluid (CSF), and conjunctivae, is often challenging.

In these cases, the selective media used for genital cultures should not be used. Blood and synovial fluid should be cultured on enriched broth media. Synovial fluid, conjunctival discharge, and CSF should be plated directly onto supplemented chocolate agar and placed in a CO_2 incubator as soon as possible. Gram's stain of conjunctival discharge, joint fluid, and CSF may be a useful adjunct.

In gonococcal septicemia (disseminated gonococcal infection—DGI), sensitivity of blood culture, under the best conditions, is often less than 50%. However, the presence of positive blood cultures often correlates with the type of DGI syndrome. For example, in cases of dermatitis and/or synovitis (without frank arthritis), half of the patients have documented bacteremia.

Gram's stains of urethral and cervical exudates should be performed when specimens for culture are obtained. In men, demonstration of typical gram-negative diplococci within polymorphonuclear leukocytes in a urethral smear is 95% sensitive and almost 100% specific.[29] In women, sensitivity of cervical smears is only a little better than 50%, although specificity is 95%. Gram's stains from rectal and pharyngeal sites are not recommended because of low sensitivity and specificity.

Susceptibility testing is not routinely performed. However, direct susceptibility testing using either the disk diffusion method or E-test diffusion testing should be performed on all isolates where initial treatment failure can result in severe sequelae (e.g., DGI, endocarditis, ophthalmia, and invasive soft-tissue infections).

3.4.2. Immunologic and Molecular Diagnostic Methods. Since the late 1980s, there has been a trend toward the use of nonculture methods. These methods offer practical and logistical advantages, such as simple specimen transport, but are expensive and must be done in a CLIA (Comprehensive Laboratory Improvement Act)-certified laboratory.

Leukocyte esterase dipsticks were initially evaluated as a cheap, initial screening test for gonorrhea. The poor sensitivity of the test (40–70%) has limited its clinical value.[30] However, it may be useful in low (<5%) prevalence settings where its high negative predictive value (93–98%) can be used to identify clients who should be assessed with culture and/or confirmatory testing, thereby focusing diagnostic resources where they will yield the highest return.[31]

Nucleic acid hybridization tests, which detect *N. gonorrhoeae*-specific DNA or RNA sequences, were developed in the early 1990s.[32] However, the relatively low sensitivity of direct hybridization tests, as compared with culture (approximately 85%, depending on site), limits their usefulness, and they largely have been replaced by nucleic acid amplification tests (NAATs). Direct hybridization tests remain useful for confirmation of culture results and/or NAAT results.

NAATs that are currently cleared for diagnostic use by the Food and Drug Administration rely on polymerase chain reaction, ligase chain reaction, strand displacement amplification, or transformation-mediated amplification.[11,33,34] The sensitivity and specificity of a NAAT depends on the nucleic acid sequence that is being amplified, the genetic characteristics of prevalent strains, which will vary over time and among different networks of people who practice unsafe sex (molecular epidemiology), and the prevalence of commensal *Neisseria* that may have acquired gonococcal nucleic acid sequences by lateral transfer, which is common among *Neisseria.*[35] In settings in which the prevalent gonococcal strains can be detected by a NAAT, sensitivities usually are greater than culture, particularly for endocervical sites, and approach 100%. Because of the high sensitivity of the DNA amplification process, these tests can be used on urine from either men or women.

Testing of oropharyngeal and rectal specimens with NAATs is not recommended because of high prevalences of pharyngeal carriage of nongonococcal *Neisseria* and interference by nucleic acid from enteric bacteria. The sensitivity and specificity of NAATs can be improved by the use of two different NAATs or by confirmation with a direct hybridization test, but this increases the cost. Efficiency can be gained by pooling urine specimens, particularly in low-prevalence settings.

4. Biological Characteristics of the Organism

N. gonorrhoeae is a fastidious aerobic and facultatively anaerobic gram-negative diplococcus that grows best at 35–36°C on a rich medium (such as chocolate agar) containing hemoglobin and a variety of other nutrients, in a moist atmosphere containing at least 3% CO_2. Characteristic colonies are small, rounded, and glistening gray after 24 hours of incubation and are nonhemolytic on blood agar.

In the early 1960s, a relationship between gonococcal colonial morphology and virulence was established. Sharply bordered colonies, which contain organisms with pili, caused urethritis when instilled into the distal urethrae of male volunteers, whereas diffuse colonies, formed by organisms without pili, were unable to cause disease following intraurethral challenge.[36] Non-piliated gonococci also are unable to invade cultured human genital epithelial cells.[37,38] Pili are thin, proteinaceous hair-like fimbriae that extend from the cell wall and enable gonococcal adherence to the epithelial cells of mucosal surfaces. Besides pilin,

the gonococcal cell membrane contains lipooligosaccharides (LOS) and three integral major outer membrane proteins (OMP).[39]

LOS are triantennary OM glycolipids that share structural organization and oligosaccharides with human glycosphingolipids (GSL).[40–42] They have oligosaccharides of three glycoform series (Table 1)—globosyl, paraglobosyl, and gangliosyl—that are linked to basal lactose disaccharides, designated Lc_2, and that extend from either or both of the basal region heptose (Hep) residues, designated Hep1 and Hep2. Hep1 is linked to Kdo; Hep2 to Hep1. The glycoform series are identical to those of the analogous GSL series.[43–45]

The organism must express paraglobosyl LOS in order to cause disease.[46] LOS have endotoxin activity similar to that of the lipopolysaccharides (LPS) found in the walls of enteric gram-negative bacteria. Gonococcal LOS induce apoptosis of ciliated human fallopian tube epithelial cells through the induction and secretion of tumor necrosis factor-α by adjacent non-ciliated epithelial cells to which the organisms bind and on which they form microcolonies.[47] The non-ciliated cells are protected from apoptosis by abundant intracellular galectin-3, a human lectin that binds β-galactose molecules that terminate LOS oligosaccharides and disrupts apoptotic pathways.[48] The loss of ciliated epithelial cells results in scarring of the fallopian tube and infertility.

Protein I, the major gonococcal OMP, consists of two porins, designated PorA and PorB, that form voltage-dependent aqueous channels that allow the selective passage of solutes through the hydrophobic outer membrane.[49] A variety of serologically distinct forms of protein I exist and are genetically stable over time. This stability has allowed the development of a monoclonal antibody-based serological typing system.[50,51]

Protein II, or opacity-associated protein (Opa), is involved in gonococcal adhesion to mucosal surfaces. The antigenic diversity and rapid antigenic variation of Opa may allow the gonococcus to escape from the immune system.[52] Protein II is closely associated with protein I in the cell membrane. The precise function of protein III is unknown, but the presence of antibodies directed against protein III may increase the risk of gonococcal infection.[53]

N. gonorrhoeae must scavenge environmental iron in order to survive and replicate.[54] Gonococci that are recovered during menses are more likely to have their phase variable hemoglobin receptor in the "on" phase than those isolated later during the cycle.[55] Most bacteria acquire iron from the environment by excreting siderophores. Gonococci, however, use a unique iron capture system by which cell surface proteins remove iron directly from human iron-binding proteins, such as lactoferrin and transferrin. This ability to steal iron from the host's iron-sequestering system is an essential factor in gonococcal pathogenesis. Gonococci are most likely to be recovered from the uterine cervix just before and during menstruation (days –2 to 5),[56,57] when hemoglobin iron is abundant.[58] Gonococcal mutants that do not express the transferrin receptor are unable to infect the male urethra.[59]

5. Descriptive Epidemiology

Useful data on the trends of reported cases of gonococcal infection are available in countries where most cases are seen in public specialized clinics (United Kingdom), where most laboratory tests are performed in a single central laboratory (Denmark), or where few changes in reporting habits have occurred (United States). In developing countries, accurate assessments of gonorrhea and other STI incidence and prevalence largely do not exist.

5.1. Prevalence and Incidence

The reported cases of uncomplicated gonococcal infection in the United States are depicted in Figure 1. The total number of reported cases peaked in 1978, with 1,013,436, and has since decreased to 330,132 in 2004. Changes in cases

Table 1. LOS Oligosaccharide Glycoform Series

Series	Designation	Structure
	$Gb_3(P^k)$	Galα1→4Galβ1→4Glc1→Hep
Globosyl	$Gb_4(P)$	GalNAcβ1→3Galα1→4Galβ1→4Glc1→Hep
	$Gb_5(P_1)$	Galα1→4Galβ1→4GlcNAcβ1→3Galβ1→4Glc1→Hep
	nLc_4	Galβ1→4GlcNAcβ1→3Galβ1→4Glc1→Hep
Paraglobosyl	(LNnT)	
	$nLc_{6–8}$	$[Gal\beta1\rightarrow4GlcNAc\beta1]_{1–2}\rightarrow3Gal\beta1\rightarrow4GlcNAc\beta1\rightarrow3Gal\beta1\rightarrow4Glc1\rightarrow Hep$
Gangliosyl	GalNAc-nLc_4	$GalNAc\beta1\rightarrow3[Gal\beta1\rightarrow4GlcNAc\beta1]_{4–8}\rightarrow3Gal\beta1\rightarrow4Glc1\rightarrow Hep$

reported among women reflect not only cases presenting for treatment but also additional case-finding by screening. Trends in acute gonococcal urethritis in men are less influenced by efforts of health workers to find cases.

Despite the decrease in the number of reported cases of gonorrhea, there has been only a minimal change since 1998 in the rate of gonococcal infections among the highest risk age groups (15–44 years old), especially in urban areas. Since the late 1990s, gonorrhea rates in homosexual men have increased dramatically,[60,61] which is especially troubling, and is part of a general trend of "behavioral disinhibition" in this risk group. These trends contrast with the sharp decrease of rectal gonorrhea in the early 1980s, representing a response to safer sex messages of the early HIV epidemic.[62]

Reported cases help define trends, but they largely represent symptomatic cases seen in public health clinics. Population-based surveys, such as those described above in Section 3.3, have defined that standard surveillance data underreport disease incidence by at least 70%.[17,63]

Serious forms of gonococcal infection, such as ophthalmia, acute and recurrent salpingitis, epididymitis, and DGI, have not been systematically recorded even in those countries, such as the United Kingdom and Scandinavia, where centralized registries are available.

5.2. Epidemic Behavior and Contagiousness

The spread of gonorrhea in a community is related not only to sexual behavior and the risk of transmission of the disease on exposure, but also to the natural course of the disease, the health behavior of infected individuals, and health care access and available resources.

Figure 2 shows the relationship of incubation period, symptoms, and transmission rates to the spread of gonorrhea; it should be useful in correcting several myths about the spread of this disease. It is incorrect to assume that all men with urethral gonorrhea develop typical acute urethritis,[18,64,65] that the vast majority of women with gonorrhea have no symptoms, or that the cycle of transmission goes from asymptomatic women to symptomatic men and back again. Tracing the course of gonorrhea in Figure 2 will illustrate these points. Begin with uninfected women being exposed to infected men: the majority of men who infect women have no or very early urethral symptoms. The effectiveness of male-to-female transmission is high. At least 50% of women will be infected during a single episode of vaginal intercourse with an infected man; risk increases to over 80% with multiple exposures to the same infected partner.[66,67] As is the case with men, women increase their risk of infection by having sex with multiple partners. Once infected, many women will develop either acute salpingitis (20–40%) or a constellation of less-specific symptoms (20–30%) (e.g., dysuria, perineal syndrome, vaginal discharge and/or odor, abnormal uterine bleeding) within 3–45 days of infection.[68,69] The remaining 30–60% of infected women will have minimal or no symptoms, yet will carry the gonococcus endocervically for 3–12 months.

Next, consider the uninfected men shown in the lower right corner of Figure 2 who will be exposed mainly to women who are unaware of symptoms; transmission of gonorrhea occurs 20–25% of the time.[70,71] Although the majority of men with urethral infection develop frank urethritis (80%), some have atypical signs and symptoms (15–18%). The tendency to seek treatment for either typical or atypical presentations of gonorrhea will vary among different social and psychological groups, so that the perceived incubation period may well vary from 2 to 30 days, if the patient seeks medical advice at all. Some asymptomatic urethral carriers may be of long duration (2–5%) and are very important vectors of gonorrhea; thus, the cycle repeats itself.

Most gonococcal infections are transmitted from asymptomatic individuals or from those who do not recognize atypical signs and symptoms of the disease. This is true for both sexes. Thus, both men and women who seek care for acute symptomatic urethritis or symptomatic pelvic infection will in all but rare cases have acquired their infection from infected sexual partners who have no symptoms. Last, infected women frequently have symptoms that are nonspecific, and some men have no symptoms. These asymptomatic men accumulate in high-risk populations and may be major contributors to the spread of disease in the community.

Homosexual men can develop symptomatic proctitis with anorectal infection, but the majority are asymptomatic. The course of urethral infection in gay men is similar to that in heterosexual men. Pharyngeal gonococcal infection is usually asymptomatic in both sexes[72] and, although quite common among gay men, is an unlikely but possible source of disease transmission.

5.3. Geographic Distribution

Reported case rates of gonorrhea vary widely among the states. These variations may reflect population factors, such as socioeconomic status, as well as differences in local control programs, such as case-finding activity, reporting practices, and the availability of public clinics.

The highest gonorrhea rates in the United States occurred in the Southeast. Large cities generally had significantly higher rates than suburban and rural communities, although gonorrhea rates in some rural areas, especially the

South, can be deceptive—although case counts may be relatively low, because of a small denominator population, rates can exceed those of the large urban areas.[73]

Although the gonorrhea rates determined for large populations are helpful in delineating trends, the "core group" theory may be more valuable for directing control efforts.[24,74] Gonorrhea rates appear to be extremely high in geographically localized, small populations participating in high-risk behavior.[24,74,75] Inner-city areas with low socioeconomic status and poor health care access are most severely affected. For example, out of a total of 202 census tracts in Baltimore, 12 census tracts located in the inner city (core) accounted for 50% of reported gonorrhea morbidity[76,77] (Figure 3). Within these census tracts, the gonorrhea rate is estimated to be five to six times higher than the overall citywide rate. Control efforts focused on core groups would potentially have the most cost-effective impact on overall gonorrhea rates.

5.4. Temporal Distribution

A marked seasonality of reported cases has been found ever since data began to be collected in the United States. Consistently, the incidence of reported disease is at least 20% higher in August through October than in February through May. The seasonality of reported gonorrhea cases may reflect combinations of greater mobility, leisure time, and sexual activities in summer. However, probably more important is the increased use of antimicrobials for upper respiratory infections during winter, which has the collateral effect of treating asymptomatic gonococcal and chlamydial infections.[78,79]

5.5. Age and Sex

Young adults and adolescents are at the greatest risk of acquiring gonorrhea. The high rates in these age groups may be secondary to increased risk-taking behavior and to physiological factors, such as increased cervical ectopy in adolescent women. During the last decade, the age group with the highest rate of gonorrhea has become progressively younger. Currently, teenage females (15–19 years of age) have the highest rate of gonorrhea. Although young adult males (20–24 years of age) have a higher rate than teenage males (15–19 years of age), the difference between these two groups has decreased markedly since 1987.

5.6. Race

Although the reported cases of gonorrhea in all ethnic groups have been decreasing since 1986, the rate of gonorrhea among African-Americans has increased 100-fold than in other ethnic groups. There is no reason to suspect an increased biological susceptibility. Traditional explanations have included lack of health care access among racial minorities and increased use of public sector clinics, which are more likely to report cases than the private sector. However, large prospective prevalence studies which have adjusted for class[17,18] clearly indicate that the racial disparity is real. Furthermore, carefully performed studies from London and Leeds in the United Kingdom, which controlled for social class and where health care access is universal, demonstrated similar consistent patterns, where Blacks of Caribbean or African backgrounds had gonorrhea rates >10 times that of whites.[80,81] The risk profile of individual patients in these studies does not differ greatly between the gonorrhea-impacted and non-impacted groups. These patterns are also seen with other STDs with wide distribution such as herpes and chlamydia.

One approach toward explaining this phenomenon has been the concept of social networks.[82,83] In the social approach, the risk profile of the *partners* is as important or even more important than the risk for the index case. Using large national datasets, social science researchers have consistently shown that African-Americans with gonorrhea are more likely to have sexual partners which are *dissociative*(i.e., different in social class and behaviors) than other groups and are more likely to have closer social/sexual network connectivity with persons at higher risk for STDs such as gonorrhea.[84–86]

5.7. Occupational and Socioeconomic Factors

Commercial sex workers are at considerably higher risk[87,88] than the general population. Gonorrhea rates of 5–10% are seen frequently in incarcerated prostitute populations. In developing countries, gonorrhea and other STDs are often associated with occupational classes that afford mobility and access to sex workers. Specific high-risk situations include truck drivers[89,90] and workers who are separated from their spouses or regular partners for long periods of time, such as migrant workers or South African gold miners. In these latter situations, there is often opportunity for development of a commercial sex industry.

5.8. Homosexuality

Rectal gonorrhea is used by public health authorities as a sentinel infection for risk behavior in homosexual men. STDs in gay men reflect social changes and societal attitudes. Gonorrhea in gay men increased dramatically in the late 1970s, concordant with the gay liberation movement,[91] and

decreased rapidly during 1981–1984 secondary to behavioral changes in the face of the AIDS epidemic.[62] Unfortunately, since 1998, there has been a resurgence which has been observed both in the United States and in Western Europe.[60,61] Hypotheses for this trend include "prevention fatigue" among gay men who have been practicing safer sex for >20 years, a rebellion of a younger generation of gay men against the safer sex peer norms of the "older" generation. However, the most likely hypothesis of this behavioral disinhibition phenomenon is the development of effective antiretroviral therapy, which has made HIV evolve from a fatal infection of short duration to a chronic, manageable disease.

5.9. Gonorrhea and HIV

Gonorrhea and other STDs facilitate HIV transmission. A Baltimore case–control study of HIV seroconverters found a 3-fold increased risk in patients who had a diagnosis of gonorrhea at their last STD clinic visit prior to seroconversion.[92] Prospective studies in India[93] and Zaire[94] conclusively demonstrated an association between exudative STD and HIV seroconversion. In the Zaire study, commercial sex workers with chlamydia had a 4.6 relative risk for seroconversion, for those with gonorrhea, the relative risk was 3.6; both relationships were stable after controlling for other confounding variables. From a population perspective, gonorrhea and chlamydial infection may be more important because they may be associated with a higher attributable fraction of HIV seroconversion cases. For example, the Baltimore investigators, whose population included a large number of intravenous drug users, concluded that the adjusted attributable fraction of HIV seroconversions related to coexistent gonococcal infection was 18%.

5.10. Antimicrobial Resistance

The emergence of plasmid-mediated penicillinase-producing *N. gonorrhoeae* (PPNG) in 1976 quickly ended penicillin's role as the treatment of choice for gonorrhea. Currently, third-generation cephalosporins and fluoroquinolones are considered the drugs of choice. However, the ever-increasing development of resistance among all pathogenic organisms in the face of antimicrobial pressure raises serious doubts about the long-term effectiveness of even these newer agents.

5.10.1. Penicillin and Beta-Lactam Resistance. Resistance to penicillin may be either chromosomally (CMRNG) or plasmid (PPNG) mediated. Chromosomally mediated increases in the minimal inhibitory concentration (MIC) of penicillin were noted during the 1950s and 1960s. By 1979, the recommended dose of penicillin was 200-fold higher than that used after World War II. However, these moderately resistant strains still had MICs < 1 μg/ml and could be successfully treated with penicillin.[95] CMRNG strains with high-level resistance (MIC > 2 μg/ml) were first identified in North Carolina in 1983.[96] Resistance in these strains resulted both from the cumulative effect of multiple genetic mutations leading to altered outer membrane permeability to antibiotics and also from changes in the penicillin-binding proteins. Due to the changes in permeability, CMRNG strains are also frequently resistant to tetracycline and second-generation cephalosporins.

Plasmid-mediated PPNG was introduced into the United States in 1976.[8] In 1992, 11.6% of isolates submitted in a sentinel survey in the United States were PPNG; however, this had decreased to 0.6% in 2004 with the decreased use of penicillin class antibiotics.[1] Interestingly, although occasional cases of cephalosporin-resistant organisms are reported,[97] chromosomally mediated resistance to cephalosporins has been rare during this period despite the increased use of ceftriaxone and similar drugs.

5.10.2. Tetracycline-Resistant Gonorrhea (TRNG). Tetracycline is not used as a sole drug for treating gonorrhea, but is recommended as a cotreatment regimen for chlamydia and is therefore widely used. Tetracycline resistance also may be either chromosomally or plasmid mediated. Plasmid-mediated TRNG strains carry a 25.2 MDa plasmid, which carries the *tetM* gene. This genetic mechanism is of public health interest for two reasons. First, it represents acquisition of a resistance determinant from other genital flora. Second, in vitro studies have been able to transfer *tetM* to other *Neisseria*, including the meningococcus.[98,99]

5.10.3. Fluoroquinolones and Resistance. Fluoroquinolones act by disrupting bacterial DNA synthesis through binding and subsequent inhibition of specific members of the topoisomerase family of enzymes, DNA gyrase and DNA topoisomerase IV,[25] which mediate the uncoiling of DNA during replication.[26,27] Primary mechanisms of resistance in GC are associated with point mutations within the *gyrA* and *parC* genes, which code for the DNA gyrase and topoisomerase IV proteins, respectively.[100–103] Efflux pump mechanisms, which mediate the active transport of quinolones from the organism after absorption, have also been described.

Quinolones were recommended as a single dose, oral therapy for gonorrhea in 1989, and became widely used in the early 1990s. Quinolone-resistant strains (QRNG) were first identified in 1991 and currently represent nearly 20% of isolates in Hawaii and California and among homosexual

men.[104–106] As a result, this class of drugs is rapidly losing its usefulness.

6. Mechanisms and Routes of Transmission

The major mode of transmission of gonococcal infection in adults is through sexual intercourse (see Section 5.2). Nonsexual transmission of gonorrhea is extremely rare among adults and epidemiological data implicating fomites in transmission are lacking. Infants born to infected mothers can acquire conjunctival infection and orogastric colonization before delivery (with premature rupture of fetal membranes) or during delivery. Children up to the age of 5 years rarely acquire the disease from fomites or inoculation with infected adult secretions. Therefore, in all infected children, family situations must be examined closely, because sexual abuse, frequently involving a member of the household, is a far more likely mode of transmission.[107] Most cases of gonorrhea in adolescent children are acquired through sexual intercourse.

7. Pathogenesis and Immunity

7.1. Pathogenesis

N. gonorrhoeae is an obligate parasite of humans that has no other natural host. In the absence of an experimental animal that is naturally susceptible to gonococcal infection investigators have returned repeatedly over the past 100 years to experimental infections of male volunteers for studies of pathogenicity and resistance.[36,108,59,109] Human challenge studies overcome the problem of not being able to reproduce faithfully in organ or tissue culture the complex molecular environment of the male urethra. They have contributed greatly to an understanding of some of the clinical parameters of gonococcal infections in men and of the infectious phenotype(s) of the organism for the male urethra.

7.1.1. Pathogenesis for the Male Urethra. *N. gonorrhoeae* cannot survive outside the human body. The reservoir of infection is within the endocervical epithelial cells of chronically infected women from which organisms are shed intermittently into the overlying mucous blanket,[57,110] where they may encounter the heavily sialylated stratified columnar epithelium of the distal male urethra.[46,111] Following exposure, men develop symptoms of dysuria and urethral discharge after incubation from 1 day to more than 1 month.[112] Most men are symptomatic within 72 hours of infection. During the period between exposure and the onset of dysuria, organisms often cannot be recovered from urine (eclipse phase) and are thought to be within urethral epithelial cells[113] from which they emerge as "infectious units" that are taken up by phagocytes that have been evoked by inflammatory cytokines released from the infected epithelial cells.[114] The release of cytokines causes dysuria, and this symptom usually is followed within a few hours by the advent of a purulent urethral discharge (leukorrhea)[46] that consists of epithelial cells and numerous polymorphonuclear leukocytes (PMN), a relatively few of which contain gonococcal microcolonies of considerable size.

The efficiency of female-to-male transmission is a function, in part, of the number of organisms that are being shed from the partner's cervix at the time of exposure (inoculum effect). It is not known, however, whether men who are exposed during the perimenstrual period are at greater risk of acquiring infection[56] than those exposed during mid-cycle, when gonococci are sparsely shed.

Resistance to infection of the distal male urethra can be overcome by increasing the inoculum in experimental infections, but most women apparently are not shedding an inoculum sufficient to overcome the level of resistance of their partner's distal urethral epithelium at the time of intercourse. The incubation period is a function of the inoculum in experimental infections in men; not surprisingly, smaller inocula take longer to produce an exudative infection than larger ones.[109]

7.1.2. Pathogenesis for the Female Endocervix. Women whose male partners are infected are exposed to a higher gonococcal inoculum than men, and male-to-female transmission of gonococci is two to four times as efficient as female-to-male transmission. Men with gonorrhea ejaculate an average of 7×10^6 (6.9×10^4–6.1×10^7) cfu in their semen.[115] This inoculum is independent of semen volume, unaffected by urination prior to ejaculation, delivered close to the endocervix, and nearly 100 times larger than that encountered by the distal male urethra during vaginal intercourse with an infected partner. The incubation period is hard to define in women, because cervical infections are often asymptomatic.

Productive infection of endocervical epithelial cells involves four distinct and sequential stages: (1) adherence, (2) uptake, (3) intracellular survival, and (4) exocytosis. Each of these uses different bacterial organelles and involves different host cellular response elements.

N. gonorrhoeae shed by infected men at the onset of leukorrhea are piliated, express Opa, and make $nLc_{4–8}$ paraglobosyl LOS molecules.[46,116] All three are needed for efficient infection of cultured genital epithelial cells.[117]

Uptake of gonococci by endocervical cells is "parasite directed" through processes that are remarkably complex.[118] Following stage 1 adherence, mediated by pili, Opa, and

an unidentified complex at the tips of host cell microvilli, the organisms replicate over several hours to form surface microcolonies. During this period, both bacteria and eukaryotic cells activate transcription and surface expression of new ligands and receptors.[119] After 4–6 hours of microcolony formation, during which microvilli adhere to bacteria only at their tips, the microvilli elongate, engage the microcolonies along their full lengths, and pull the cell membrane around the microcolony until it is fully engulfed (stage 2).

The onset of symptoms in men with experimental gonorrhea is heralded by a switch in the Opa phenotype of shed organisms from Opa^- to Opa^+.[46] Individual Opa variants can bind up to four different CEACAM receptors. CEACAM are a family of cellular adhesion molecules that are variously expressed by cells in different tissues. Cells of many tissues express more than one CEACAM; other cells express none. CEACAM-mediated uptake of gonococci by endocervical epithelial cells appears to result in the death of the organism; however, CEACAM-mediated uptake by PMNs may account for the survival of the microorganisms within these phagocytes.[120] Only about a third of women constitutively express CEACAM on their endocervical epithelial cells. Since roughly a third of women resist gonococcal infection after exposure,[70] constitutive CEACAM expression may enable endocervical epithelial cells to resist infection.

The terminal β-gal of nLc_{6-8} LOS molecules is a ligand for two human lectins that have been implicated in gonococcal pathogenesis, the asialoglycoprotein receptor (ASGPR), a C-type lectin, and galectin-3, an S-type (secreted) lectin.[48] Gonococcal adherence to genital epithelial cells causes upregulation of ASGPR expression, but not that of galectin-3. Galectin-3 is a polyfunctional molecule that participates in inflammatory responses and is integral to terminal differentiation processes. Epithelial cells that are invaded by gonococci constitutively express galectin-3. Ligation of galectin-3 by nLc_{6-8} LOS molecules may prevent apoptosis and enable intracellular survival (stage 3).[121,122] Unsubstituted paraglobosyl LOS bind C3b to their terminal galactose[123]; bound C3b then is degraded to iC3b, the primary ligand for the CR3 complement receptor, which is constitutively expressed by endocervical cells. How—and when—in the processes of productive infection the nLc_{4-8} LOS:iC3b:CR3 complex acts remains to be determined.

Endocervical epithelial cells express the lutropin (luteinizing hormone) receptor (LHr). Gonococcal adherence to uterine cells causes the bacteria to express a neo-ligand that engages the LHr after 4–6 hours on the cell surface.[124] This results in the uptake of microcolonies. The mucosal epithelium of the female lower genital tract consists of columnar cells that line the endocervix and squamous cells that line the endocervix and vagina; the latter do not support gonococcal uptake. Unlike the epithelial cells that line the urethra, endocervical epithelial cells do not respond to gonococcal attachment and invasion with the release of inflammatory cytokines.[125,126] The lack of an endocervical cytokine response explains the absence of symptoms in most infected women.

7.1.3. Disseminated Gonococcal Infection (DGI). In over 98% of cases, gonococcal infection is restricted to the mucosal epithelium of the male urethra (local infection) or the female genital tract including the uterus and fallopian tubes (ascending infection). This restriction is accomplished by complement-mediated lysis of serum-sensitive bacteria as they enter the bloodstream. In about 1% of cases, mucosal infection, often asymptomatic, will progress to septicemia, caused by variants of the infecting strain that have altered LOS that do not bind the IgM and IgG antibodies which circulate in most people's serum, and that initiate immune lysis. Patients with defects in terminal complement components are at higher risk for DGI, as well, as they cannot complete the lytic cascade.[127–129]

7.2. Immunity

Antibody responses during uncomplicated cervical infections are modest as compared with those that would be expected during a gram-negative infection of the urinary tract.[125,126] IgG is the predominant Ig isotype in endocervical secretions. IgA is produced locally by the endocervix, is overwhelmingly (>80%) polymeric sIgA, and is equally distributed between its two isotypes. IgG and IgM, on the other hand, originate mainly as serum immunoglobulins that are transudated into the endocervical canal. Gonococcal IgM is present in cervical secretions only during the first 2 weeks after infection; gonococcal sIgA is found reliably in cervical secretions during infection, but its levels decline rapidly with successful treatment and are undetectable 50 days after infection.[130,131] Reinfection occurs only after sIgA levels in cervical secretions have waned. Given the short duration of the IgM and sIgA responses after gonococcal infection and the absence of immunological memory of prior infections, women who are only intermittently exposed to the organism are unlikely to have appreciable levels of either Ig in their cervical secretions at the time of exposure. In contrast with sIgA, gonococcal IgG levels in cervical secretions mirror those in serum, arising 7–10 days after infection and declining slowly thereafter. As a result of their different decay kinetics, IgG predominates over sIgA in endocervical secretions.

Gonococcal serum IgG antibodies are acquired with each infection, so that over time and repeated infections, levels of resistance increase.[132,133]

7.2.1. Protein Antibodies. IgG antibodies that bind pilus and OMP are more abundant in the sera of previously infected individuals, and bind lower molecular weight proteins, than in the sera of never-infected individuals, but such antibodies are found readily in the sera of men and women before, or at the time of, a diagnosis of gonococcal infection. Pili are highly immunogenic, but pilus antibodies, which often are present in the sera of infected individuals at diagnosis, do not prevent infection. A purified pilus vaccine that consistently induced broadly cross-reactive IgG antibodies failed to prevent gonorrhea in a field trial that involved 3,250 volunteers.[134]

The porin proteins do not consistently induce antibodies during infection, but may contribute to protection after repeated exposures.[132]

Opa are highly variable, and the antibody response to them during infection is complex. Opa antibodies are not abundant prior to infection and do not appear to protect against endocervical infections. They may provide some protection against PID, however.[107]

Protein III antibodies may actually increase the risk of infection by interfering with complement-dependent clearance of the organisms.[53]

7.2.2. Lipooligosaccharide (LOS) Antibodies. Increases in LOS antibodies—and induction of new LOS specificities—are readily detected in serum and urine during naturally acquired and experimental gonococcal infections.[46,109,135] Paraglobosyl LOS IgG antibodies, induced during a first experimental infection, are associated with resistance to a subsequent challenge. Paraglobosyl LOS IgG is induced during natural urethral infection of men, but also by colonization by commensal *Neisseria*, including *N. meningitidis*, which also express these LOS molecules.

Paraglobosyl LOS IgG are bactericidal for gonococci isolated from local, mucosal infections; that is, they initiate complement-mediated lysis. Gangliosyl IgM also circulates in most people by early adolescence, and these antibodies also are bactericidal for most strains.

8. Patterns of Host Response

Gonococcal infection may be limited to mucosal surfaces, such as the urethra, cervix, pharynx, eyes, and rectum, or, in a significant proportion of infected women, may ascend along the uterine endometrium to the fallopian tubes (PID) or escape immune lysis, such as in DGI.

8.1. Clinical Features

8.1.1. Urethritis in Men. Dysuria and purulent discharge are the most common symptoms.[111,136,137] Although most men have one or both symptoms within 1 week of infection, 5–10% of cases remain asymptomatic.

The differential diagnosis of urethritis in men is between gonococcal urethritis (GCU) and nongonococcal urethritis (NGU). Although GCU is more frequently associated with purulent discharge and dysuria, the diagnosis cannot be confirmed or excluded by history and physical examination alone. Gonorrhea is diagnosed presumptively by Gram's stain of a urethral smear and confirmed by culture or nucleic acid-based tests (Section 3.4). Coinfection with *Chlamydia trachomatis* is common and occurs in up to 50% of patients in some areas.[138]

Untreated gonococcal urethritis usually resolves within 6 months. Because the vast majority of symptomatic men seek early treatment, there are few current epidemiological data on the long-term sequelae of untreated gonococcal urethritis. In developing countries, where chronic untreated infections still occur, paraurethral and preputial abscesses, fistula formation, and urethral strictures occur frequently. Epididymitis and prostatitis are manifestations of ascending infections in men.

8.1.2. Endocervicitis. In most women with gonococcal cervicitis, purulent endocervical exudates and cervical erythema and edema can be detected by a careful visual speculum examination. Erosive cervicitis usually is present only with *C. trachomatis* coinfection, which is common.[112,139] Infected women may have non-specific complaints, such as increased vaginal discharge, abnormal menses (increased flow or dysmenorrhea), dyspareunia, or dysuria. Patients often do not associate these symptoms with an STD. Labial tenderness and Bartholin's and Skene's gland abscesses are less frequent signs of infection. Fifty percent of women are asymptomatic at presentation. Bimanual examination of the pelvis is mandatory in evaluating women with gonococcal infection. As many as half of women with endocervical gonorrhea may at initial evaluation have upper-tract signs such as adnexal tenderness, if such signs are carefully sought.[3,69,140,141]

Definitive diagnosis of gonococcal cervicitis is made by culture or nucleic acid-based tests. Gram's stain of cervical secretions may be useful in identifying cases at the bedside, but has a sensitivity of only 50–70% (see Section 3.4). Coinfection with other genitourinary pathogens, such as *C. trachomatis* or *Trichomonas vaginalis*, is common. Because of the particularly high proportion of coinfection by *C. trachomatis* and the relatively high cost of diagnosis, treatment regimens must be directed against both pathogens.

8.1.3. Upper Genital Tract Infection and PID. PID is the most important complication of gonorrhea. Ascending infection from the endocervix through the uterine cavity

to the fallopian tubes usually occurs within one to two menstrual cycles in 10–20% of women with untreated or undertreated gonococcal infection.[69,140,141] PID is actually a spectrum of soft-tissue infections, including endometritis and salpingitis. Leakage of infected material can result in tubo-ovarian abscess and/or pelvic peritonitis. Apoptotic loss of ciliated tubal epithelial cells impairs ciliary motility, setting the stage for superinfection with vaginal flora and secondary abscess formation. Tubal scarring, the major long-term sequelae of PID, leads to increased susceptibility to repeated episodes of PID as well as to an increased incidence of tubal infertility and ectopic pregnancy.[142]

Patients at highest risk for developing PID are under 25 years old, have had previous episodes of PID, have multiple sexual partners, and douche. Use of oral contraceptives as a risk factor for PID is still a subject of considerable debate.

Clinical diagnosis of PID traditionally has been made on the basis of the triad of lower abdominal pain, abnormal cervical or vaginal discharge, and tenderness on bimanual examination. Fever occurs in only half the cases. Women with PID may have crampy, dull, lower abdominal pain occurring with menses or irregular menses over several cycles before the disease becomes overt. On bimanual examination, the signs of PID are uterine traction tenderness, adnexal tenderness, or adnexal enlargement. Although adnexal abscesses are unusual in gonococcal PID, clinical suspicion should lead to further evaluation, such as sonography. The wide spectrum of disease, including mild cases, and the relative non-specificity of clinical signs make accurate diagnosis difficult. Laparoscopic studies have demonstrated clinicopathological correlation in only two thirds of cases. Therefore, careful clinical observation of the patient is extremely important.

Because of the diagnostic problems and the profound sequelae of untreated or undertreated disease, the CDC and other authorities recommend an aggressive approach to PID diagnosis and treatment.[140] Treatment regimens for PID are designed to include adequate antimicrobial coverage for *N. gonorrhoeae*, *C. trachomatis*, gram-negative organisms, and anaerobes.[143] Hospitalization may be necessary in severe cases. In addition, if foreign bodies, such as IUDs, are present, they should be removed.

8.1.4. Anorectal Infections. Anorectal gonorrhea occurs in homosexual men with a history of receptive rectal intercourse.[144,145] A small proportion of women with endocervical gonorrhea have coexistent rectal infection.[144] In women, anorectal gonorrhea can occur even in women who have not had rectal intercourse. In these cases, infected perineal secretions are thought to secondarily infect the anorectal mucosa.

Most anorectal infections in women are asymptomatic. In homosexual men, symptoms such as tenesmus, mucoid discharge, hematochezia, perianal irritation, and constipation are seen more frequently. Anoscopy in symptomatic cases may show erythema, exudates, and friable mucosa in the anal canal and terminal rectum. In asymptomatic cases, however, anoscopy is often normal. The differential diagnosis of anorectal gonorrhea, especially in homosexual men, includes rectal herpes and *C. trachomatis*. Diagnosis is most accurately made by culture.

8.1.5. Pharyngeal Infection. Pharyngeal infection usually results from oral sexual exposure to an infected partner.[72,146,147] Among patients with anogenital gonorrhea, pharyngeal infection has been demonstrated in some studies in 3–7% of heterosexual men, 5–20% of women, and 10–25% of homosexual men. Among pregnant women, the proportion may be higher. Although exudative pharyngitis and sore throat may occur, pharyngeal gonorrhea is most commonly asymptomatic. Sore throat in some patients may be more related to a post-fellatio syndrome than to an infectious process. Asymptomatic pharyngeal infection resulting in DGI has been well described.

Diagnosis of pharyngeal gonorrhea should be made by culture because of the high prevalence of commensal *Neisseria* which cross-react with other diagnostic tests.

8.1.6. Disseminated Gonococcal Infection. Approximately 0.5% of patients with gonorrhea develop DGI. Patients with DGI have a local infection, rectal, urogenital, or pharyngeal, that is often asymptomatic. Host and organism factors are both important in permitting dissemination. As described earlier, organisms from patients with DGI are more likely to express LOS phase variants, rather than paraglobosyl or gangliosyl molecules, and to be serum resistant.[127,128,148,149] Organisms that cause DGI are generally more sensitive to antibiotics than those found in routine anogenital infections. DGI occurs more commonly in women than men; pregnant women are at even higher risk, probably because of suppressed immunity. Persons with terminal complement deficiencies also are at very high risk for dissemination and may experience multiple episodes.

DGI can be separated clinically into two syndromes. The first syndrome is a dermatitis-arthritis. Patients present with fevers, chills, polyarthralgias, or tenosynovitis. Joint and tendon symptoms are not migratory, but additive. Three to twenty petechial, papular, pustular, hemorrhagic, or necrotic skin lesions (frequently in combination) may be present on the distal extremities. Fingers, toes, hands, and wrists are commonly involved in an asymmetrical fashion. Blood cultures are positive for *N. gonorrhoeae* 50%. The second common syndrome is a septic arthritis, usually

monoarticular, affecting large joints. The knee is involved most commonly. Although the septic arthritis is secondary to a bacteremic process, blood cultures at this stage are rarely positive. Culture-negative cases are thought to result from either immune complex deposition or intra-articular killing of the organisms prior to arthrocentesis. DGI generally responds rapidly to appropriate antibiotic therapy. In a small proportion of patients with DGI, severe and potentially fatal complications such as meningitis or endocarditis[9] can occur.

8.1.7. Gonococcal Ophthalmia. Gonococcal ophthalmia occurs in neonates born to women with endocervical gonorrhea and adults who are exposed to infected secretions.[150,151] Adult gonococcal ophthalmia is occasionally seen in young adults with anogenital gonorrhea. Accidental autoinoculation is thought to be the cause of many cases. Purulent keratoconjunctivitis is seen clinically.

Neonatal gonococcal ophthalmia is rare in developed countries because of prenatal screening and universal prophylaxis.[152,153] In developing countries, however, gonococcal ophthalmia is still a major cause of blindness.[154] Clinically, diffuse redness and conjunctival swelling are seen first, followed by a profuse, purulent discharge from which the organism can be easily identified by Gram's stain and culture. Corneal scarring and blindness may occur if the infection is left untreated.

Neonatal gonococcal ophthalmia can be prevented by maternal screening and treatment, where indicated. In the United States, routine prophylaxis is given at birth with erythromycin, silver nitrate, or tetracycline. Tetracycline is currently preferred because of less irritation.

8.1.8. Pediatric Gonorrhea. Gonococcal infections in prepubertal children[152] should be considered evidence of sexual abuse unless proven otherwise.[107,155] Because of forensic implications, specific diagnosis is imperative. The chain of custody of cultures must be maintained, cultures from all sites should be confirmed by experienced laboratories, and positive specimens should be archived. In young girls either a symptomatic or an asymptomatic vulvovaginitis may be seen. Urethritis has been described in boys, and anorectal, rectal, and pharyngeal infection may be seen in both sexes.

8.2. Diagnosis

Infection with *N. gonorrhoea* should be included in the differential diagnosis of urethritis, cervicitis, septic arthritis, conjunctivitis, and sepsis in sexually active adults. Most pharyngeal infections with *N. gonorrhoeae* are asymptomatic. However, *N. gonorrhoeae* should be considered as a possible etiology of acute pharyngitis in patients with a history of orogenital sexual exposure. Newborns with conjunctivitis may be infected with *N. gonorrhoeae*.

A specific diagnosis of *N. gonorrhoeae* can usually be made by either Gram's stain or culture. The application of the Gram's stain and culture to specific gonococcal syndromes is detailed in Section 3.4.1. During the last decade, there have been major advances in nonculture diagnostic methods. Molecular diagnostic tests for *N. gonorrhoeae* are covered in Section 3.4.2.

9. Control and Prevention

9.1. Principles of Control

For control through public health intervention, we emphasize the importance of establishing priorities among gonorrhea cases for the implementation of measures such as intensive case-finding and epidemiological treatment. In other words, public health authorities must attempt to identify gonorrhea cases where intervention will have the highest yield in preventing further spread (see Sections 9.1.2 and 9.1.3). The mistaken assumption that every case of gonorrhea is equally important for the further spread of the disease must be dispelled. Failure to treat gonorrhea cases and to promote primary prevention among groups with a high rate of disease transmission significantly limits the chances of success. Different degrees of control are possible in different environments. Control programs in many developed countries are currently focused on efforts to decrease the prevalence of the disease. In other areas, attempts may be made to prevent only serious sequelae, i.e., gonococcal ophthalmia neonatorum and gonococcal PID.

9.1.1. Clinical Services. Provision of easily accessible clinical services and proficient laboratory support services are essential to control programs. Clearly, it is futile to engage in widespread screening and elaborate efforts to locate sexual partners, while waiting periods in clinics are long, patients with symptoms are being turned away, and the misdiagnosis and mismanagement of known cases are common. The establishment of practical clinical services should precede attempts at more sophisticated intervention and may in itself have a significant effect on the control of gonorrhea in some communities. To increase accessibility, all services related to the control of gonorrhea should be rendered free of charge, or at least not denied because of an inability to pay.

The prevention of gonococcal ophthalmia neonatorum is perhaps the only substantial intervention that is not rooted in STD clinical services. Even ophthalmia prophylaxis requires trained clinical personnel. Community education and promotion of primary prevention (e.g.,

abstinence, partner selection, and personal prophylaxis) should be based on epidemiological information derived from clinical facilities.

9.1.2. Epidemiological Process. Because of the large reservoir of asymptomatic and minimally symptomatic cases, especially in women, gonorrhea cannot be controlled solely by the application of diagnosis and treatment to cases with apparent symptoms. Public health intervention is targeted both at identifying potentially infected individuals and in preventing the further transmission of disease.

Direct disease control activities are usually a function of local health departments. Ideally, the epidemiological process should focus on five groups: new patients, sexual partners of patients, patients with repeated infections, health care providers, and community sources of health information. The basic information to be obtained from patients is directed toward identifying potentially infected partners and should include age, race, sex, place of residence, mobility, and special sociobehavioral features (e.g., prostitution, employment). Health care providers for the population at highest risk must be determined. Simple measures for doing so include (1) finding out from patients and their sexual partners where they are seeking health care; (2) making a survey of health care providers located near the places of residence of high-risk groups; and (3) seeking "hidden" health care providers (e.g., pharmacists, "street health providers", hotel physicians). In addition, a sample of physicians should be visited to find out the extent of their services to gonorrhea patients, if there is reason to suspect that a significant proportion of cases are not being reported. Health care providers serving high-risk groups must be assisted in the correct diagnosis and treatment of gonorrhea and encouraged to use appropriate intervention techniques.[156] For example, acute detention facilities (e.g., city jails) have been found to have extraordinarily high rates of disease.[157,158] Therefore, this may be a setting to increase diagnostic and treatment services.

Each community may have unique sources of health information. The quality and influence of these sources (peers, media, social groups, churches, opinion leaders) will vary for subgroups within the community. Liaison with these information sources should be sought by local health authorities.

9.1.3. Transmitters or Nontransmitters. Special emphasis must be placed on finding those who are infected. Patients who are most likely to be efficient transmitters of the disease deserve special emphasis in case-finding activities. Generally, efficient transmitters are those who are either asymptomatic or tend to ignore symptoms, who have a large number of different sexual partners, and who have limited access to medical care. A good indicator of a group with a high proportion of transmitters is the rate of repeated infection in the group. Efficient transmitters have also been linked epidemiologically to the core group (see Section 5.3).

9.1.4. Diagnostic Screening. Because of cost considerations, culture screening for case detection in gonorrhea control must be targeted to high-risk groups. The cost-effectiveness of screening will depend on the prevalence of the disease in the population and can be increased by concentrating on high-risk groups. However, although this measure will find cases at a lower cost per case, control effectiveness will depend on the proportion of existing cases missed by the screening procedure and other control strategies.

Populations screened should be carefully selected. For example, women presenting with signs or symptoms of other STDs, women distinguished by behavioral or demographic factors characteristic of high-risk groups (e.g., prostitutes), and women from groups in which the prevalence of the disease is high should be those selected for initial screening. The concept of repeated screening may be applied in a retrospective or prospective fashion. Retrospectively, women and men with a history of gonococcal infection should be screened whenever they visit a health facility. Prospectively, most authorities believe that immediate posttreatment evaluation (test of cure) is low yield. However, because of the high reexposure rate, it may be prudent to rescreen in 2–3 months. For example, careful studies of patients with gonorrhea have found that 10–15% will become reinfected within 3 months.[159]

Available resources will determine the number of screenings and the combinations of initial and repeat screenings that are possible within each locality. In most circumstances, only women are screened for gonorrhea. However, NAATs, which can be performed on urine, make noninvasive testing in men practical (see Section 3.4). In high-prevalence areas, screening in different clinical settings should also be considered (e.g., in antenatal, gynecological, or family planning clinics). Endocervical culture is the preferred screening method in women.

9.1.5. Epidemiological Treatment. Epidemiological treatment consists of providing full therapeutic doses of antibiotics to persons recently exposed to gonorrhea, while awaiting the results of laboratory tests to confirm a diagnosis. This practice is recommended because of the significant chance that the patient has been infected, the serious consequences of the disease, the relative safety and efficacy of treatment, the frequency with which people in high-risk groups default on appointments, and the risk of the spread of the disease in the community.

9.1.5.1. Counseling to Obtain the Simultaneous Treatment of Sexual Partners. Every gonorrhea patient should

receive counseling with the aim of ensuring that (1) the patient will return for reexamination; (2) the patient will advise his or her sexual partners to seek examination and treatment; (3) the patient will abstain from sexual contact until the sexual partners have been examined and treated; and (4) should the patient be reinfected in the future, he or she will bring recent sexual partners along to the health care facility.

9.1.5.2. More Intensive Case Management. These techniques range from brief motivational educational sessions with individual patients to systems using referral cards and to actual personal interviews with patients that will enable public health workers to visit the patient's sexual partners in the community and offer treatment. Individual personal interviews to learn the names and whereabouts of sexual contacts and, in particular, community visits by health workers to locate these contacts may be possible in some areas. Whatever methods for providing epidemiological treatment to patient's sexual partners are used, they may not need to be used uniformly for all patients.

9.1.5.3. Priorities for Epidemiological Services. The aim of contact tracing is to provide epidemiological treatment for those most likely to have transmitted the disease recently and most likely to continue transmitting it in the future. Contact tracing also requires the accessibility of the index case to the counselor or public health worker. Granting this, priority for epidemiological services should be given to these groups of patients, symptomatic men and members of high-risk groups (Table 2). When the sexual partners of these groups of patients are examined, the rate of asymptomatic infection is high. If the control organization is still weak, initially it may be most practical for epidemiological services to concentrate on men with symptomatic gonococcal urethritis.

9.1.6. Health Education. Health education has been widely accepted as an important tool in the control of STDs because of increased recognition of the behavioral and sociocultural factors involved in their prevention, transmission, diagnosis, and treatment. Medical and organizational measures by themselves have proven to be unsatisfactory. In some countries, sex and health education is neglected, particularly among the young. The consequent ignorance about STDs may hinder their control because infected patients may be slow to take advice. Decisions and action to adopt control and preventive measures in relation to STDs depend on several factors, such as information and misconceptions about these diseases, changes in beliefs about them and in attitudes toward them, prevailing cultural views and social norms, and the way patients are received by the STD health providers.

Health education activities may be aimed at making the public aware of the STD problem in the community, at seeking the active cooperation of groups and individuals in control activities, at informing groups at risk about control and preventive measures and motivating them to adopt these measures, at educating young people to consider the health-related aspects of their sexuality, at preparing personnel for their educational functions in the control program, and at orienting services at the relevant clinics toward the needs of their clients.

9.1.7. Partner Management. One of the most important interventions in STDs has been partner management. This has been particularly challenging, especially in environments where partners are not accessible. This can be particularly difficult in areas where commercial sex workers or gay men with anonymous sex partners are operative. Another challenge, especially in the management of gonorrhea and chlamydia infections, has been in accessing partners, especially in areas where there have been poor resource availability. For example, a variety of studies have demonstrated that interventions are much more effective than traditional self-referred partner management strategies; however, resources are difficult to access. The traditional approach has been health department outreach, where trained disease intervention specialists bring individuals identified as sexual contacts of infected patients into the clinic for evaluation and treatment, or alternatively, assure referral. An alternative approach has been providing *partner-delivered therapy.* Under this approach, an individual partner receives therapy which he/she would then give to his/her partner at home .[160,161] This approach has been utilized in a number of cities, but faces regulatory challenges. The regulatory challenges include liability issues (for example, the practitioner is providing therapy for an individual who is technically not the practitioner's patient), reimbursement issues, especially when both partners are not part of the same health plan, and regulatory issues from pharmacy and medical boards. This approach also requires a level of trust between sexual partners. Nevertheless, these barriers have been overcome in a variety of states. The most

Table 2. Examples of Priority Criteria for Disease Intervention[a]

Prostitutes
Women with pelvic inflammatory disease
Symptomatic men
Cases of treatment failure (possibility of resistance)
Residence within a defined "core" area
Antimicrobial-resistant infection
Use of "crack" cocaine
Large number of sexual contacts

[a]These are examples of criteria used by different communities. Not all may be applicable to any one community.

important study in addressing partner management issues was conducted by Golden. He demonstrated that provision of partner therapy either through patient-delivered therapy or provision of partner therapy through a network of referral pharmacies substantially reduced gonorrhea reinfection in the index patients. These data, although fairly conclusive, however, represent an enormous investment of resources, which may not be applicable to most areas.

9.2. Macro-Level Epidemiological Approaches

Designing control strategies also requires assessing the impact or sociogeographic effects. STDs predominantly affect specific demographic subgroups. Within the broad population strata, STDs are often concentrated in areas where poverty and other limitations on health care access exist. This has resulted in development of the "core group" concept.[24,162–164] The core groups are population subgroups which are disproportionately impacted by STDs and which often act as the endemic reservoir of disease within a community. These may be either sociologically or geographically defined. For example, in developing countries, the predominant core groups are long-distance truck drivers and commercial sex workers, whereas in the United States, there is often a direct link to drug use. On a community level, gonorrhea is often associated with other indicators of social disruption, such as a high number of liquor stores and abandoned buildings.[165,166]

In the United States and Western Europe, core groups are often defined geographically as residents of defined core areas which typically are socioeconomically depressed. In the US urban environment, core areas have been well documented for gonorrhea and other STDs such as syphilis and HIV. Within a core area, disease incidence may be 20–30 times that of surrounding areas. For example, in Baltimore we have identified discrete core areas where the reported gonorrhea rate in 1994 for persons aged 15–39 years was 6,821/100,000 for men and 4,341/100,000 for women and areas where primary and secondary syphilis incidence had similar disproportionately high rates.[25,167]

The transmission efficiency of gonorrhea and syphilis through unprotected sexual exposure has been empirically estimated to be 50–70% per exposure and treatment effectively breaks the transmission chain. Therefore, under most circumstances, introduction of gonorrhea into a community free of infection should not result in a sustained epidemic. Community-based models of STD transmission therefore postulate that core areas or epidemiologically defined core groups are critical to maintaining high rates of gonorrhea. In these models, cores are characterized by a high transmission density, an empirical function that is dependent on characteristics of the sexual network. In contrast to many other infectious diseases, STD transmission is dependent on behavior, i.e., sexual intercourse. STD transmission is therefore limited to sexual networks, i.e., the interrelated sexual connections of a defined social group.

9.2.1. Social Networks. Traditional STD intervention approaches have used partner notification as a major tool in control programs. As discussed earlier, for diseases like gonorrhea—where the incubation period is short—or diseases with prolonged latency periods (asymptomatic infection) that is efficiently transmitted—such as chlamydia—partner notification may not work. In contrast, syphilis has been traditionally controlled with partner notification using the rationale of the "critical period". As a control tool, however, partner notification has never been fully and formally evaluated. In settings where partners are not named—such as in drug-related prostitution—the process fails. New approaches have utilized understanding the social network,[168,169] i.e., those individuals who are associated with the infected individual. These approaches appear to be successful in identifying additional infected persons.

9.3. Personal Prophylaxis

Condoms, when used appropriately, are highly effective in preventing transmission of gonorrhea and other STDs.[170–173] Latex condoms are recommended, since natural membrane condoms contain pores that may allow the transmission of small viral particles, such as HIV. These pores are not present in latex condoms. Although there is substantial current work on microbicides, those tested to date have not been shown to be effective in preventing incident gonococcal infection.[174]

9.4. Possibilities of Immunization

The LOS, OMP, and pili of *N. gonorrhoeae* have been viewed as potential candidates for vaccine development.[109,116,134] Vaccine development has been complicated by the lack of an animal model, antigenic diversity among strains, and antigenic variation within strains over time.

The failure of OMP and pilus vaccines has focused attention on LOS, particularly paraglobosyl LOS. Seroepidemiological surveys and resistance to experimental infection provide compelling support for the development of a paraglobosyl LOS vaccine, but formulating such a vaccine will be a daunting undertaking, given the endotoxic

properties of LOS and the molecular mimicry of human paraglobosyl glycosphingolipids by paraglobosyl LOS. It is unlikely that a simple, effective vaccine can be developed in the near term, although technical advances may lead to success in the future.

10. Unresolved Problems

The gonococcus has historically demonstrated great versatility and adaptability. In the next few years, it would not be unreasonable to expect the continued development of antimicrobial resistance. Quinolone resistance appears to be particularly ominous.

However, we may also expect to see a continued decrease in gonorrhea incidence because of improved diagnosis, which in turn will enhance control. The new NAATs are a major advance that facilitate noninvasive screening. From a practical standpoint, gonococcal NAATs often are combined with *Chlamydia* NAATs. However, NAATs are more expensive than traditional tests and require advanced laboratory resources and investment.

Finally, advances in glycobiology and immunology may finally point the way toward effective local preventive measures for gonorrhea and other STDs and make possible the development of a safe and effective gonococcal vaccine.

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CHAPTER 17

Haemophilus influenzae

Janet R. Gilsdorf

1. Introduction

Haemophilus influenzae, a common commensal of the pharynx of humans, is considered part of the normal pharyngeal flora. In addition, it is an important cause of human infections. Strains that possess one of the six polysaccharide capsules, types a through f, cause invasive infections such as bacteremia, meningitis, septic arthritis, epiglottitis, and facial cellulitis, with type b infections being significantly more common (greater than 90%) than types a and c–f. Use of the highly successful type b vaccine, composed of the type b polysaccharide conjugated to one of several protein carriers, has resulted in dramatic reductions in rates of invasive infections in the United States and in many Western countries. Strains with no capsule, the so-called non-typeable strains, are frequent causes of localized infections such as otitis media and sinusitis in children and adults, as well as bronchitis and pneumonia in adults. While the organism lives asymptomatically in the pharynges, it does not cause localized pharyngitis. No vaccine is yet available to protect against infection with non-typeable strains.

2. Historical Background

H. influenzae are gram-negative, pleomorphic bacilli originally described by Pfeiffer in 1892.[1] Known as the Pfeiffer bacillus, it was isolated from patients with influenza and assumed to be the causative agent of what is now known to be viral influenza.[2] Ultimately its association with influenza was recognized to be secondary rather than causal. Subsequently it was named *Haemophilus influenzae,* referring to its absolute heme requirement for growth (heme = blood, philus = loving) and its historically presumed role in influenza. In 1931, Margaret Pittman classified *H. influenzae* by the presence of one of six antigenically distinct polysaccharide capsules (types a–f), based on the ability of the bacteria to be agglutinated by capsule-specific typing antisera.[3] Strains that lack capsule (non-typeable) are not agglutinated by any of the typing antisera.

In the past century, *H. influenzae* has been at the center of a number of important scientific discoveries. Fothergill and Wright, in 1933, recognized the association of protection against *H. influenzae* meningitis with the level of bactericidal activity of blood, thus describing the role of age-related immunity in protection against infection with encapsulated bacteria.[4] In 1980, Smith and colleagues described the mechanism with which DNA is taken up by *H. influenzae*.[5] This observation explained at the molecular level the process of transformation of DNA by competent bacterial cells. In the early 1980s, Porter Anderson[6] as well as John Robbins and Rachel Schneerson[7] pioneered the techniques of conjugating protein carrier molecules to the *H. influenzae* capsular polysaccharide polyribosyl ribitol phosphate (PRP). This technology is now used in the preparation of conjugated polysaccharide vaccines that prevent *H. influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis* infections. Finally, in 1995, *H. influenzae* strain Rd was the first free-living organism to undergo DNA sequence analysis of its entire genome,[8] ushering in the era of complete genome analyses of bacteria with the resultant increased understanding of bacterial pathogenesis, physiology, and genetic variability.

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3. Methodology

3.1. Epidemiologic Reporting

Surveillance of invasive *H. influenzae* infections in the United States has been conducted by the National Notifiable Diseases Surveillance System (NNDSS, http://www.cdc.gov/EPO/DPHSI/nndsshis.htm) which is operated by Centers for Disease Control and Prevention (CDC) in collaboration with the Council of State and Territorial Epidemiologists (CSTE) and published weekly in MMWR. Data include weekly and annual national cumulative statistics on type b, non-type b, and unknown serotype infections in children <5 years old (Table I) as well as weekly and annual regional and state by state data for invasive *H. influenzae* infections of all serotypes in all ages (Table II, Part 2) and are available on the CDC Web site from 1996 to the present (http://www.cdc.gov/EPO/DPHSI/annsum/index.htm).

In addition, surveillance of *H. influenzae* invasive infections is conducted by the Active Bacterial Core (ABC) surveillance system, a component of the CDC's Emerging Infections Program Network (EIP), a collaboration between CDC, state health departments, and universities, consisting of 10 sites across the United States, representing a population of over 38 million persons (http://www.cdc.gov/ncidod/dbmd/abcs/survreports.htm). Data collected include invasive infections caused by type b, non-b, non-typeable, and unknown types stratified by age groups. Cases of meningitis and bacteremia without focus, with their respective mortality rates, are reported.

Non-invasive *H. influenzae* infections are not reportable in the United States.

3.2. *H. influenzae* Typing Methods

Epidemiologic studies of *H. influenzae* infections require a system of clustering the causative organisms in a meaningful way. A number of typing systems have been used to group strains as alike or different based on phenotypic characteristics such as capsular type, biotype, outer membrane protein type, or multilocus enzyme electrophoresis (MLEE) type. More recently, a number of molecular typing systems have been used to cluster *H. influenzae* strains based on restriction fragment length polymorphisms (RFLP), polymerase chain reaction (PCR) fragment sizes, and DNA sequences of various regions of the *H. influenzae* genome.

3.2.1. Phenotype-Based Typing Methods

3.2.1.1. Capsule and Capsular Typing. Classical serotyping requires specific reagents and skilled, experienced laboratory technologists and interlaboratory variability in typing results is unacceptably high using this technique.[9] Thus, newer molecular amplification methods have been developed[10] that utilize PCR amplification of the capsular gene regions. One set of primers is used to amplify the *bexA* region, which is required for export of the capsule to the cell surface and is conserved in all encapsulated *H. influenzae* strains. Recent data demonstrating allelic variation in *bexA* among types a, e, and f strains suggest that detection of this gene will be improved with an additional primer set.[11,12] A second series of primers, which amplify the type-specific domain of the *cap* gene region of typeable strains, are used to determine the specific capsular type, a–f. Allelic variants of *pgi*, which encodes phosphoglucose isomerase, cluster with specific *H. influenzae* serotypes, suggesting *pgi* genotyping may serve as a marker for serotype.[13]

3.2.1.2. Biotyping. *H. influenzae* have been classified by biotype on the basis of three biochemical activities—the production of indole and the presence of urease and ornithine decarboxylase activities.[14] Eight biotypes have been identified, see Table 1; most clinical isolates are biotypes I, II, or III. While specific biotypes have been associated with virulent strains,[15] this technique is not discriminatory enough to be useful in clustering *H. influenzae* for meaningful epidemiologic investigations.

3.2.1.3. Outer Membrane Typing. Early attempts at sub-typing *H. influenzae* used outer membrane protein profiles, generated by digesting the bacterial outer membranes with detergents or by sonication and separating the outer membrane fragments by gel electrophoresis.[16,17] This technique was used to track transmission of *H. influenzae* strains[16] and to compare commensal strains with otitis media strains from the same subject.[18] Outer membrane typing has been replaced by newer molecular-based methods for identifying related *H. influenzae*.

3.2.1.4. Multilocus Enzyme Electrophoresis (MLEE). MLEE differentiates *H. influenzae* strains based on the electrophoretic pattern differences of 15–17 metabolic enzymes.[19–21] In the largest study of *H. influenzae* electrophoretic types among encapsulated strains (mostly type b)

Table 1. Biotype Characteristics of *Haemophilus influenzae*

H. influenzae biotype	Indole production	Urease activity	Ornithine decarboxylase activity
I	+	+	+
II	+	+	–
III	–	+	–
IV	–	+	+
V	+	–	+
VI	–	–	+
VII	+	–	–
VIII	–	–	–

280 types were identified.[19] Type b strains were found to fall into two major phylogenetic groups, division I (in which strains exhibit duplication of the *cap* gene region, which is flanked by IS*1016* elements) and the much smaller division II (in which strains have a single copy of the *cap* gene region, are not flanked by IS*1016* elements, and the *cap* region may be located in a different chromosomal site from Division I strains[22]). Further MLEE analysis showed that non-typeable strains are phylogenetically distinct from type b strains and more heterogeneous.[23] MLEE is costly and time intense to perform and has largely been abandoned.

3.2.2. Molecular-Based Genotyping Methods. The classical typing systems to characterize groups of *H. influenzae*, capsular typing and biotyping, are limited by their ability to identify only seven serotype groups (including non-typeable) and eight biotype groups. Recently, molecular typing systems have been developed to expand the ability to group *H. influenzae*. Each molecular typing system relies on various genetic alterations that give rise to polymorphisms in the *H. influenzae* genome and distinguishes strains by characterizing different molecular parameters of the strains (Table 2). Thus, strains grouped as similar by one technique may not group as similar by another.

For restriction fragment-linked polymorphism (RFLP)-based methods, such as pulsed field gel electrophoresis (PFGE), genomic DNA is digested with rare-cutting restriction enzymes and the resulting DNA fragments are separated by gel electrophoresis and the patterns analyzed. In a related RFLP-hybridization method (such as ribotyping) genomic DNA is digested with frequent-cutting restriction enzymes and separated; resultant DNA fragments are then interrogated with gene-specific nucleic acid probes. For polymerase chain reaction (PCR)-based typing methods (such as the random primer methods RAPD and ERIC as well as repetitive element PCR [rep-PCR] techniques), specifically designed primers hybridize to the complementary genomic DNA and the intervening DNA sequences are amplified; the resultant DNA amplicons are then separated by gel electrophoresis and the patterns analyzed. For DNA sequence-based methods (such as 16S rRNA typing, multilocus sequence typing (MLST), and multilocus variable number tandem repeat analysis (MLVA)), primers are designed to amplify the region of interest, the intervening DNA sequences are amplified, the amplicons are subjected to DNA sequence analysis, and the sequences are compared.

The ideal typing system is capable of distinguishing apparently unrelated strains from each other but is not so discriminatory that each isolate is designated a unique type. Any system must be validated using epidemiologically related and unrelated strains and must test strains passed in the laboratory several times to assess the stability of the typing parameters. The results of typing by molecular-based methods may differ dramatically in the number of unique patterns observed among various *H. influenzae* strains, reflecting increased clonality among type b strains compared to non-typeable strains, and in the definition of unique genotypes.

When molecular-based typing methods that utilize electrophoretic banding differences are used to analyze large numbers of strains, the resulting patterns are often analyzed by computer-based cluster analysis methods in which a Dice coefficient matrix of pairwise differences in the pattern profiles is constructed and the pattern divergences calculated from this matrix are displayed in a dendrogram constructed by the unweighted pair-group method using arithmetic averages (UPGMA).[24] While these distance (phenetics) methods accurately display clustered strains, they are not excellent methods of displaying phylogenetic data to make

Table 2. Molecular-Based Typing Methods of *Haemophilus influenzae*

Methods	Discriminator	Type based on
RFLP		
PFGE	Sizes of restriction fragments	Patterns of bands on a gel
PFGE + hybridization	Sizes of restriction fragments that hybridized to gene-specific probes	Patterns of bands on a gel
PCR		
RAPD™, ERIC™, RepPCR	Sizes of PCR-generated amplicons	Patterns of bands on a gel
DNA sequence		
MLST, MLVA, 16sDNA	Nucleotide sequences of PCR-generated amplicons	Nucleotide sequence differences

RFLP = restriction fragment length polymorphism
PFGE = pulsed field gel electrophoresis
PCR = polymerase chain reaction
RepPCR = repetitive sequence PCR
MLST = multilocus sequence typing
MLVA = multilocus variable number tandem repeat analysis

inferences on evolutionary descent,[25] which are better displayed using character (cladistic) methods.[26,27]

3.2.2.1. Restriction Fragment Length Polymorphism (RFLP)-Based Typing Methods. Ribotyping: Ribotyping distinguishes bacterial strains by restriction length polymorphisms of their genetic regions that contain genes encoding ribosomal RNA. The bacterial DNA is digested with frequent-cutting restriction endonucleases, the resulting bands are separated by gel electrophoresis, and the gels are interrogated with a labeled rRNA probe.[28] DNA from 120 *H. influenzae* strains (77 were type b, the remainder were non-typeable) digested with *Hae* III and *Eco* RI yielded three and six, respectively, different ribotype groups.[29] In another study, DNA from 75 non-typeable strains digested with *Eco* RI yielded 45 ribotype groups.[30] In a study from Taiwan, ribotyping yielded 6 patterns among 55 type b strains, 9 patterns among 10 isolates of non-b-typeable strains, and 12 profiles among 22 non-typeable *H. influenzae*,[31] thus confirming increased diversity among non-typeable strains compared to type b strains. Ribotyping, while potentially useful in genotyping *H. influenzae*, was less discriminatory than pulsed field gel electrophoresis.[30]

Pulsed field gel electrophoresis (PFGE) typing: PFGE, often considered the gold standard of molecular genotyping, distinguishes bacterial strains by difference in the sizes of their restriction fragments. The strain DNA is digested with rare-cutting (8 base pair) restriction endonucleases (often *Sma* I, *Spe* I, or *Eag* I are used with *H. influenzae*) and the resulting bands are separated by gel electrophoresis. The differences in banding patterns result from strain-to-strain differences in the presence and location of the restriction endonuclease sites across the *H. influenzae* genome. Characteristically 5–20 fragments are generated, ranging in size from 10 to 800 kb in length. See Figure 1.

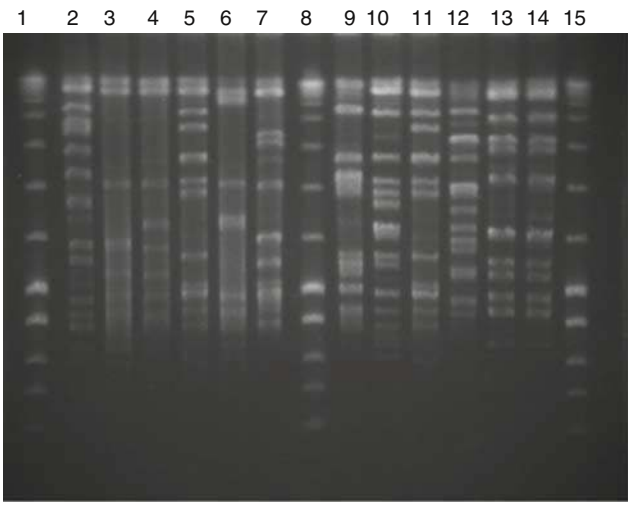


Figure 1. Patterns of bands on a gel: PFGE of DNA of *Haemophilus influenzae* isolated from children in a day care center and digested with the restriction enzyme *Sma* I. Lanes 1, 8, and 15 = fragment size markers. Strains in lanes 5 and 11 and in lanes13 and 14 are genotypically identical.

RFLP typing strategies were originally designed to identify related organisms during outbreak of infectious diseases, and standard guidelines for interpreting the DNA restriction patterns generated by this technique for outbreak investigation have been developed.[32] Computer simulation digestion of bacterial DNA by restriction enzymes demonstrated that errors associated with both band determination and band matching contributed to variable PFGE results. Use of multiple enzymes improved the fidelity between the PFGE results and the true sequence similarities.[25,33]

PFGE has been used to document relationships between *H. influenzae* strains isolated from children attending the same day care,[34,35] between strains serially isolated from the same individual,[36] between strains from household contacts of patients with invasive *H. influenzae* infections,[37,38] and between non-β-lactamase-resistant *H. influenzae*.[39,40] PFGE has shown the limited diversity of type a strains isolated during outbreaks in Alaska and on the American Indian reservations[41,42] and the absence of change in population structure of strains isolated before and after introduction of the Hib vaccine.[43,44] Repeatedly, except in outbreak situations, PFGE has shown the highly diverse phylogenetic structure of *H. influenzae*, particularly that of non-typeable strains.

3.2.2.2. Polymerase Chain Reaction (PCR) Fragments-Based Typing Methods. PCR-based methods rely on analysis of DNA fragments derived from amplification of specific bacterial genome regions, using primers that hybridize with predetermined nucleotides that are present, usually, within or flanking the genomic region of interest.

Random amplified sequences-based genome typing methods: Random amplified sequences typing (RAPD™) is a PCR-based technique in which amplicons are generated using nucleic acid primers with no specificity for *H. influenzae* DNA. RAPD using either AP1 or AP2 primer sets was more discriminatory in *H. influenzae* than ribotyping, yielding 10–13 (respectively) bands for analysis.[29] In many studies, RAPD results have been found to be labile with poor reproducibility on repeated testing of the same sample,[37,45,46] although commercial kits may reduce the poor reproducibility to some extent.[47]

Enterobacterial repetitive intergenic consensus (ERIC) typing is a PCR-based technique in which amplicons are generated using nucleic acid region primers that hybridize with many gene regions in *Escherichia coli.* These specific gene regions have been shown to be absent in *H. influenzae* so when used to type *H. influenzae*, this technique actually

depends on amplification of random sequences. ERIC, using ERIC I primers, was found to be more discriminating than ribotyping for *H. influenzae*.[30] The poor reproducibility of random amplified sequence typing may be related to the low stringency required of the amplification reaction to allow hybridization of the random primers.[30]

Repetitive element PCR typing: Repetitive element typing utilizes PCR to amplify DNA regions located between repeated sequences in bacterial genomes. The sequence of *H. influenzae* strain Rd revealed a number of repeated sequences and several of these have been used for genotyping.

Bruant et al. amplified fragments using primers containing the 23-base pair sequence commonly called intergenic dyad sequence (IDS), which is found in multiple copies throughout the *H. influenzae* genome, commonly in pairs as inverted repeats.[48] The results showed approximately 10 bands that could be used for analysis and 62 different patterns were identified among 66 non-typeable strains; epidemiologically linked strains showed identical or closely related patterns. Reproducibility using the IDS typing technique was excellent, possibility because of the high stringency conditions possible with use of the *H. influenzae*- specific primers.

The genome of *H. influenzae* strain Rd contains 1465 copies of the 9 base pair uptake signal sequence (USS) 5'-AAGTGCGGT-3'.[8] Experience with the use of these repeats in repetitive element typing has shown the generation of overly complex banding patterns because of the large number of USS repeats.[48] USS typing using different stringency conditions may have a role as an initial screen in a multi-stage typing system.[36]

3.2.2.3. Sequence-Based Genome Typing Methods. Standard gel electrophoresis-based techniques to type *H. influenzae* show considerable lab to lab variability and are subject to variations in laboratory conditions used to perform the assays. DNA sequence-based techniques, on the other hand, yield unambiguous data that may readily be shared from lab to lab.

16S rRNA typing: Sequence differences of 16S ribosomal RNA genes have formed the basis for genotyping several pathogenic bacteria, including *H. influenzae*.[49] The level of discrimination between strains was similar to multilocus sequence typing (see below), although the two methods grouped the non-typeable strains differently. *H. influenzae* possess six 16SrRNA operons that may not be identical in sequence,[8] potentially complicating sequence analysis of strains possessing differing 16S rRNA genes.[50]

Multilocus sequence typing (MLST): MLST was developed as an epidemiologic tool and has been widely used to genotype a large number of bacterial species.[51] The principle of the method relies on indexing variation in the slowly evolving core genes.[52] To genotype *H. influenzae* the MLST technique uses the sequences of seven genes that encode central metabolic enzymes ("housekeeping genes"), *adk*, *atpG*, *frdB*, *fucK*, *mdh*, *pgi*, and *recA*. Sequences of approximately 440 bp of each gene are concatenated for analysis. Each unique allele of each gene is assigned an arbitrary identifier, and the sequence types (ST) of the strains are defined on the basis of their allelic profile over the seven loci examined. An open source repository for *H. influenzae* sequences has been established, hosted at Imperial College in London and developed through funding by the Wellcome Trust (http://haemophilus.mlst.net). Over 600 strains are included in the *H. influenzae* database but are not an unbiased representation of the whole population of *H. influenzae* as strains isolated from clinical specimens are overrepresented.

The two approaches for examining the levels of relatedness between strains using MLST data include (1) clustering procedures based on pairwise differences in allelic profiles, either on a gene-by-gene basis or by concatenating the data for all seven loci, and (2) phylogenetic analysis based on allele sequences. Because the UPGMA clustering algorithm, based on allelic mismatches, may not adequately represent the pattern of events that occurred during genetic diversification, eBURST, which identifies the most parsimonious founding genotypes, has been developed to incorporate a more realistic model of clonal expansion,[53] see Figure 2.

MLST has been used to characterize the phylogenetic relationships of encapsulated and non-encapsulated *H. influenzae*.[54] Recombination was identified among the seven housekeeping genes studied (particularly evident in *pgi*, *recA*, *mdh*, and *frdB*) and appeared to be less frequent among encapsulated strains. The average number of alleles for each of the gene loci was 27, with a range of 22–36. Among 26 non-typeable isolates, 19 sequence types were identified, and with one exception, isolates with identical sequence types were epidemiologically close linked. A recent modification of MLST uses a multiplex format to amplify all seven genes in the same PCR reaction.[55]

Multilocus variable number tandem repeat analysis (MLVA): Tandem repeat regions possessing variable numbers of repeats have been identified in almost all bacterial species. The number of repeats in each repeat locus varies from strain to strain because of genetic instability of the repeat regions[56] and forms the basis for MLVA genotyping. In *H. influenzae*, short 2–7 base pair tandem repeats are extremely common in the genome and are often associated with virulence genes, where the number of repeats correlates with expression or non-expression of the virulence factor, through a mechanism termed phase variation (see Section 4.3.2.4, Phase variation). In MLVA typing, primer pairs amplify DNA regions that contain the repeat sequences,[57] the amplicons are then sequenced, and the number of repeats

will be

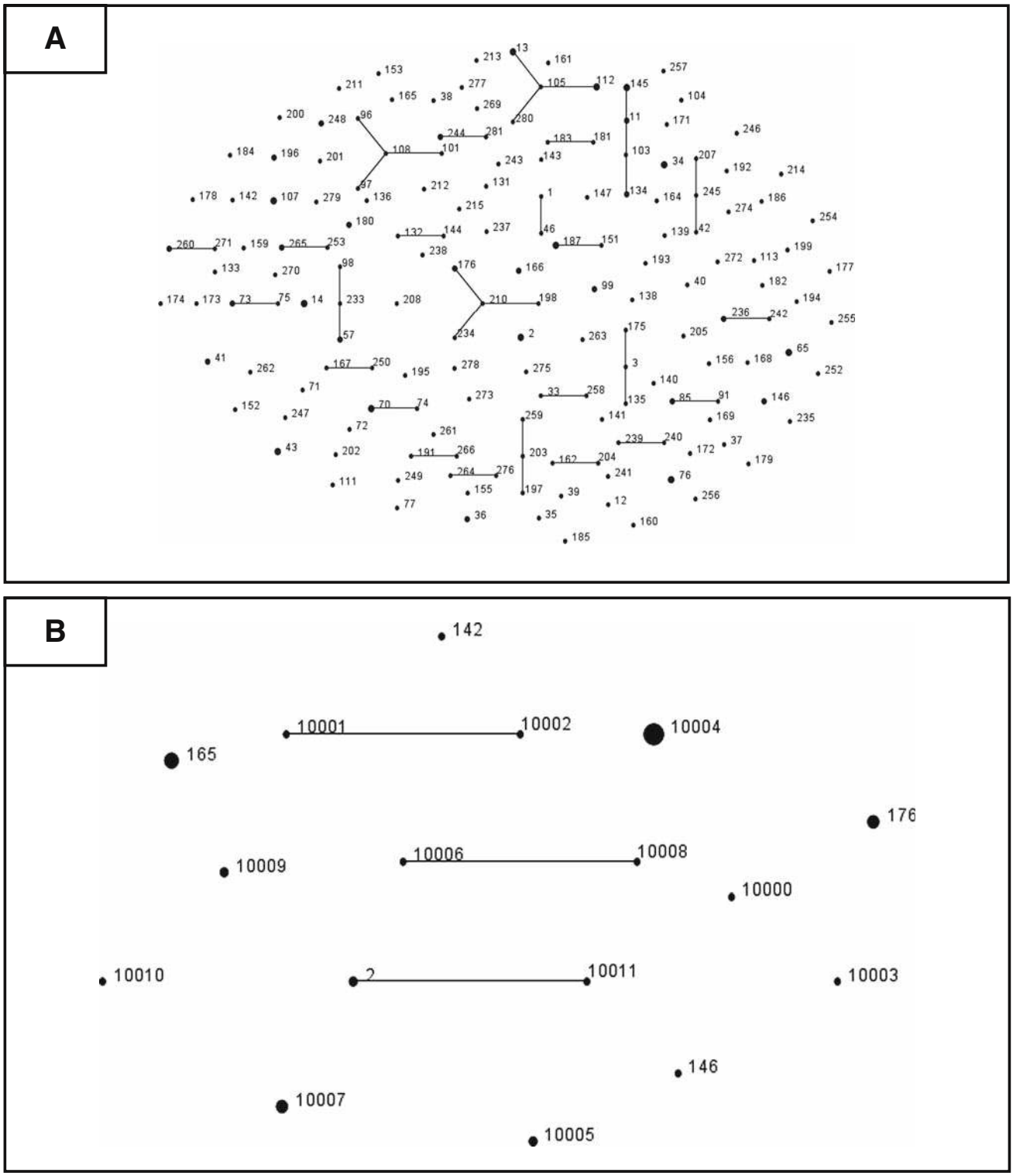


Figure 2. Multilocus sequence patterns of non-typeable *H. influenzae*, **A**, and of type b *H. influenzae*, **B**.

found in the sequence are used to establish the genotype. Further refinement of this technique has used four primer sets, to amplify areas with three or four repeats composed of sequences of 5–12 base pairs.[55] MLVA typing was found to be more discriminating for differences in type b *H. influenzae* than was MLST.[55]

3.3. Laboratory Diagnosis of *H. influenzae* Infection

The diagnosis of an infection caused by *H. influenzae* relies, first, on a clinical suspicion that these bacteria may be the causative agent of a patient's symptoms. There is no role for screening tests to facilitate early detection of *H. influenzae* infections. Positive throat or nasal cultures for *H. influenzae* do not predict an association with a specific clinical infection, as many individuals are colonized with *H. influenzae* and only a few of those colonized experience disease.

3.3.1. Culture. The most reliable diagnostic test for *H. influenzae* disease is bacterial culture of material obtained from the site of infection. These organisms are fastidious and require enriched nutrient media containing factor X (heme) and V (nicotinamide adenine dinucleotide, or NAD) for growth. The ideal laboratory growth conditions are 35°C with 5–10% CO_2. Clinical microbiology laboratories use chocolate agar to identify *H. influenzae* from specimens other than blood and automated culture systems to process blood specimens.

Most invasive *H. influenzae* infections are associated with concomitant bacteremia and blood culture may be critical to identifying the causative organism. In children, the level of *H. influenzae* bacteremia correlates with the severity of the infections, i.e., patients with $\geq$100 colony-forming units per milliliter were more likely to have meningitis.[58] Most patients with invasive type b infections have $>$15 colony-forming units per milliliter. Thus, the organisms can often be detected in samples containing relatively low blood volumes, and successful recovery of *H. influenzae* usually requires only a single, aerobic blood culture.[59] Prior receipt of an antibiotic to which *H. influenzae* are sensitive may compromise the ability to detect these bacteria by culture.

In addition to blood cultures, cultures of material obtained directly from the site of infection is important in establishing the causative organism, such an cerebrospinal fluid from patients with meningitis, joint fluid from patients with septic arthritis, and pleural fluid from children with pneumonia with pleural effusion. Blood cultures from children or adults with non-typeable *H. influenzae* respiratory tract infections such as pneumonia, bronchitis, sinusitis, or otitis media rarely reveal the causative bacteria. While no longer routine clinical practice for patients with uncomplicated acute otitis media, cultures of the middle ear fluid is the only way to diagnose *H. influenzae* acute otitis media. *H. influenzae* may also be isolated from sinus aspirates and bronchial-alveolar lavage specimens.

3.3.2. Identification of *H. influenzae*. In clinical microbiology laboratories, *H. influenzae* are characterized as gram-negative, pleomorphic bacilli that grow on chocolate agar but not sheep's blood agar and require X and V factors for growth. Factor V (NAD) requirement is assessed by bacterial growth surrounding NAD-containing discs placed on a bacterial lawn inoculated on nutrient agar plates or by satellitism around *Staphylococcus aureus* strains that secrete NAD (factor V).[60] Heme (factor X) requirement is assessed by bacterial growth surrounding heme containing discs or by failure of the colonies to elaborate porphyrin, indicating that they do not synthesize heme and, thus, require factor X.[61] *H. influenzae* are classically distinguished from the closely related, but not pathogenic, *Haemophilus haemolyticus* by failure to lyse horse or rabbit erythrocytes.

Recent studies have shown that *H. haemolyticus* often spontaneously lose hemolytic activity and, thus, appear identical to *H. influenzae* by standard classification methods. *H. influenzae* may be distinguished, however, from non-hemolytic *H. haemolyticus* by the presence of *iga*, which encodes IgA protease[60]; presence of a specific epitope in the outer membrane protein P6[62]; presence of *sodC* or its cognate protein copper–zinc superoxide dismutase[63]; and a unique amplification product following polymerase chain reaction using *H. influenzae*-specific 16S rDNA primers.[62] Although *H. haemolyticus* strains are not associated with disease, between 10 and 40% of *H. influenzae*-like isolates from the respiratory tracts of patients with chronic obstructive pulmonary disease (COPD) or of asymptomatic individuals[62,64] are non-hemolytic *H. haemolyticus*.

3.3.3. Antigen Detection. Commercial kits are available to detect the capsular polysaccharide, PRP, of type b *H. influenzae* in serum, cerebral spinal fluid, and urine and may be used to confirm type b *H. influenzae* infection.[65] Because accurate test interpretation is highly dependent on the experience of the technician, this test is not widely used in clinical microbiology laboratories.

3.3.4. Polymerase Chain Reaction (PCR). While PCR has been used to detect *H. influenzae* DNA in sterile middle ear effusions[66] and from the blood of patients with meningitis or pneumonia,[67] such nucleic acid amplification techniques are not routinely used for the diagnosis of *H. influenzae* infections.

3.3.5. Serology. Infection with type b *H. influenzae* results in stimulation of type b capsule antibodies in children over age 2 years. The development of an immune response to the capsule or to non-type b antigens, however, requires 1–2 weeks and is not clinically useful in diagnosing an acute infection with *H. influenzae* type b.

4. Biological Characteristics of *H. influenzae*

The *Haemophilus* genus resides in the Gammaproteobacteria and is a member of the family Pasteurellaceae, which also includes the genera *Actinobacillus* and *Pasteurella*.

4.1. Growth Requirements

H. influenzae are unable to synthesize protoporphyrin IX (the immediate precursor of heme, an essential factor for many bacterial metabolic functions) from δ aminolevulinic acid. Thus, *H. influenzae* have an absolute requirement for exogenous heme (also known as factor X) for growth. In addition, they require nicotinamide adenine dinucleotide, or NAD (also known as factor V), for growth. In the laboratory *H. influenzae* grow best in complex media containing factors X and V (such as chocolate agar) under aerobic or facultatively anaerobic conditions at 35–37°C. Many strains show improved growth in 5–10% CO_2.[60]

4.2. Natural Competency

H. influenzae, like the other respiratory pathogens *S. pneumoniae* and *Neisseria*, are naturally competent; they possess specific mechanisms to acquire DNA from the environment.[68] Bacterial cells made competent during stationary growth phase are able to bind and absorb double-stranded DNA to their surface through the agency of the 9 base pair DNA uptake signal sequence 5'-AAGTGCGGT-3',[69] of which 1464 copies are randomly scattered across the *H. influenzae* genome.[70] After entering the cytoplasm, the donor DNA becomes single stranded and, upon hybridization with complementary bases on the recipient DNA, is incorporated into the host chromosome by homologous recombination.[71]

4.3. Genetic Variability

In adapting to its limited ecologic niches in the human pharynx, *H. influenzae* rely on fitness selection and clonal expansion of those population members that express the phenotypic characteristics important for survival in a particular human niche.[72] The sources of selective pressure that drive genotypic, and thus phenotypic, diversity among various populations of *H. influenzae* include host immune responses,[73] nutritional resources in the various microenvironments of the human respiratory mucosa[74] or blood stream,[75] and host factors necessary for bacterial adherence,[76] cellular penetration,[76] and host-to-host transmission.[77] The following genetic mechanisms alter either *H. influenzae* gene expression or gene content and generate organisms with fitness characteristics optimal for survival under various selective pressures, thus allowing the bacteria to adapt to rapidly changing microenvironments.

4.3.1. Genome Sequence. In 1995, the DNA sequence of the full genome of *H. influenzae* strain Rd (a multi-passaged, type d strain that had spontaneously lost expression of its capsule) was reported.[8] This genome was slightly over 1.8 million base pairs in size, with 1743 predicted coding regions. Importantly, several known virulence genes of *H. influenzae* were not present in strain Rd, such as those encoding haemagglutinating pili, Hap, and the high molecular adhesins HMW. Since then, complete or partial sequence information has become available for an increasing number of *H. influenzae* strains; complete sequences are deposited in the NCBI Web site: http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi: Comparisons of the genes in these genomes reveal considerable differences in gene content between the strains; 280 open reading frames were identified in strain 86-028NP that were absent from the Rd genome.[78]

4.3.2. Mechanisms of Genetic Diversity. A major theme in the biology of respiratory pathogens, including *H. influenzae*, is antigenic diversity, and several genetic mechanisms facilitating antigenic diversity of *H. influenzae* have been elucidated, including the following.

4.3.2.1. DNA Point Mutations. Mistakes in DNA replication that result in single base pair mutations in the *H. influenzae* genome create gene polymorphisms that may alter the immunologic, and occasionally the functional, character of proteins encoded by the mutated genes. The vast majority of spontaneous DNA mutations are detrimental to the bacterial cell and do not promote increased bacterial fitness and survival.

4.3.2.2. Presence or Absence of Coding Regions. Comparison of the gene content of the sequenced NTHi strains demonstrates as much as 25% variation.[79, 80] These changes represent loss or gain of genetic loci through spontaneous deletions or acquisition of bacterial genetic material through DNA uptake and homologous recombination into the *H. influenzae* genome.

4.3.2.3. Horizontal Gene Transfer. *H. influenzae* are naturally competent and, thus, capable of taking up environmental DNA and incorporating it into the bacterial chromosome by homologous recombination. As a result, "mosaicism," the apparent mixing and matching of genetic elements from various NTHi strains, occurs. Horizontal transfer of the *H. influenzae* P2 gene (which encodes an outer membrane protein), the *fts* gene (which encodes penicillin binding protein 3), and a specific capsule locus deletion between *H. influenzae* strains has been demonstrated.[81–84]

Acquisition of DNA from non-*Haemophilus* sources results in regions of *H. influenzae* with codon usage that reflects the donor DNA rather than native *H. influenzae* DNA. Sixty so-called putative aliens, loci with significant codon usage deviations from the average *H. influenzae* genome, have been identified in the Rd genome.[85] An example of non-*Haemophilus* DNA acquisition is the intergenic lateral transfer of tryptophanase gene cluster between *E. coli* and *H. influenzae*.[85]

4.3.2.4. Phase Variation. Expression of a number of gene products in *H. influenzae* is turned off and on through the agency of tandem repeat loci (referred to as microsatellites or contingency loci) in either the promoter regions or the coding regions of the genes. While these loci may contain repeats of 2–11 base pairs, most consist of tetranucleotides[57]; between 2 and 57 repeats have been reported. Because of the instability of the repeats, slipped strand mispairing occurs, which alters the numbers of DNA repeats present. As a result, the coding region is in or out of frame, resulting in high-frequency, stochastic, heritable genotypic switching of expression or modulation of transcription.[86] Examples include the contingency loci associated with genes that encode hemagglutinating pili, lipooligosaccharide (LOS)-modifying proteins, high molecular weight (HMW) adhesins, and heme-haptoglobin binding proteins[87–89] as well as additional genes of unknown function.[57] Phase variation is thought to be driven by changes in environmental conditions[90] and is considered a mechanism for immune evasion.[91]

4.3.2.5. Hypermutability. Most bacteria in a population are constrained in their ability to mutate, and deleterious mutations are much more common than beneficial mutations. The presence of a few bacteria in a population that are capable of frequent mutations provides the population with organisms that may be suited for survival during a sudden change in environmental conditions.[86] Many hypermutable organisms have defects in genes that encode components of the methyl-directed mismatch repair (MMR) system. In *H. influenzae,* hypermutable strains were found to have a mutation in *mutS* and to be more prevalent among strains isolated from the respiratory tracts of patients with cystic fibrosis, occurring in 14.5% of isolates,[92] than from other disease states.[93]

4.3.2.6. Allelic Variation. Minor changes in DNA sequence of *H. influenzae* virulence genes may alter their function. Sequence variants of *hmwA*, which encodes the high molecular weight adhesin, demonstrate variable binding characteristics to different epithelial cell receptors.[94] In addition, variants of *lpsA* (which encodes a glycosyl transferase) have been shown to add either glucose or galactose to *H. influenzae* LOS, and variants in the 3' end of the gene direct the anomeric linkage of the added hexose.[95]

4.4. Antibiotic Resistance

Like all bacteria, *H. influenzae* may possess genes associated with resistance to antimicrobial agents commonly used to treat infections caused by this agent. The resistance of *H. influenzae* to ampicillin and other β-lactam antibiotics is generally limited to either the production of a β-lactamase, with resultant enzymatic hydrolysis of the β-lactam-containing antibiotic, or the presence of altered penicillin binding proteins on the bacterial surface that exhibit lowered affinity for β-lactam antibiotic binding. Susceptibility of *H. influenzae* to all β-lactam antibiotics is generally predicted by susceptibility to ampicillin as defined by the Clinical and Laboratory Standards Institute (CLSI) MIC breakpoints, which are as follows: ≤ 1 μg/ml = susceptible, 2 μg/ml = intermediate, and ≥ 4 μg/ml = resistant.[96]

Resistance to ampicillin emerged in the 1970s, with the recognition of TEM-1 type β-lactamases, and has increased since that time,[97] with emergence and spread of additional β-lactamases, including ROB-1 β-lactamases and uncharacterized, novel β-lactamases. Prevalence of β-lactamase-producing strains varies widely by geographical area, ranging from 3% in Germany to 65% in South Korea.[98] Resistance prevalences also fluctuate over time, with US strains showing 36% β-lactamase-positive strains in 1994 compared to 26% in 2002.[99]

Ampicillin resistance in the absence of β-lactamase production was first described in the early1980s in type b and in non-encapsulated strains.[100,101] In these β-lactamase-negative, ampicillin-resistant (BLNAR) strains, nucleotide substitutions in the penicillin binding outer membrane transpeptidase PBP3 lead to reduced β-lactam antibiotic binding to the bacterial surface,[97] with MICs of 1–4 μg/ml. The prevalence of BLNAR strains varies worldwide and is seen in 40% of strains in Japan and 10% in Europe but $<5\%$ in the United States.[102]

Because plasmid-mediated TEM-1 and ROB-1 β-lactamase genes are located on transposons (i.e., Tn1), this type of antibiotic resistance may be transmitted between bacteria. In contrast, β-lactamase-negative, ampicillin resistance has arisen spontaneously among *H. influenzae* strains, with little evidence of clonal spread,[103] except for limited spread of specific BLNAR clones in patients with cystic fibrosis, between outpatients attending a single hospital, and between parents, children, and siblings.[97]

H. influenzae may be intrinsically resistant to macrolide-lincosamide-streptogramin B agents and ketolides and tetracyclines because of the action of efflux pumps; resistant to chloramphenicol through the production of plasmid-mediated production of chloramphenicol acetyltransferase;

and resistant to trimethoprim-sulfamethoxazole because of increased production of the enzyme dihydrofolate reductase.[97] Quinolone resistance among *H. influenzae*, due to alterations in DNA gyrase or topoisomerase IV genes, has been described and an outbreak with a levofloxacin-resistant strain has been reported in a long-term care facility.[104]

5. Descriptive Epidemiology

5.1. Epidemiology of Infections Caused by Type b *H. influenzae* (Hib)

5.1.1. Classical Hib Epidemiology in Non-immune Individuals. Prior to the introduction of the Hib vaccines in 1985, Hib was the most common cause of meningitis in young children in the United States. As recorded by Fothergill and Wright in 1933[4] and continues in many non-vaccinated populations, Hib infections occur rarely in the first 2 months of life because neonates are protected by placental passage of maternal anti-type b capsular antibodies. The peak age in unvaccinated children is 6–8 months,[105,106] with approximately 50% of cases occurring prior to age 12 months. The incidence falls during early childhood, with low incidence in children older than age 24 months, when naturally occurring antibody against the type b capsule begins to appear.

Before the use of the Hib vaccines, approximately one child in 200 (incidence between 40 and 100 cases/100,000 in the United States) developed Hib disease by age 5 years.[107] Among unimmunized children within the United States, certain racial/ethnic groups are at increased risk of invasive Hib infections, including American Indians[108,109] and Native Alaskans,[110] with incidence rates between 200 and 1000 cases/100,000. These high rates may be explained by living in large households under crowded conditions.[111,112]

Hib meningitis shows a bimodal seasonal pattern, with peaks between March and May and in October and November.[107] In most studies, no gender differences in Hib infections are seen.

5.1.2. Current Hib Epidemiology, US Children. With the introduction of the conjugated Hib vaccine in 1987, the epidemiology of *H. influenzae* type b infections changed dramatically (Figure 3). Recent data confirm that few *H. influenzae* invasive infections occur in children in the United States, as documented by the Active Bacterial Core surveillance (ABCs) program sponsored by the Centers for Disease Control (Table 3), or in individuals of all ages, as documented by the National Notifiable Diseases Surveillance System (Figure 4). Because of the variable capability for accurate serotype analysis in different laboratories, some of the strains of unknown type may actually represent type b strains.[9] Shortly after introduction of the vaccine, a strong herd effect related to vaccine protection was recognized[113] due to the vaccine's ability to reduce Hib colonization with subsequent interruption in person-to-person transmission, which contributes to the reduction in infections.

Similar to data collected prior to introduction of the vaccine, the highest rates of Hib disease in US children in 1994–1995 occurred among American Indian children, followed by African American and Caucasian children. Lowest rates occur among Asian/Pacific Islander and Hispanic children[114] (Table 4). The racial/ethnic discrepancies in these rates may be related to differences in vaccine coverage

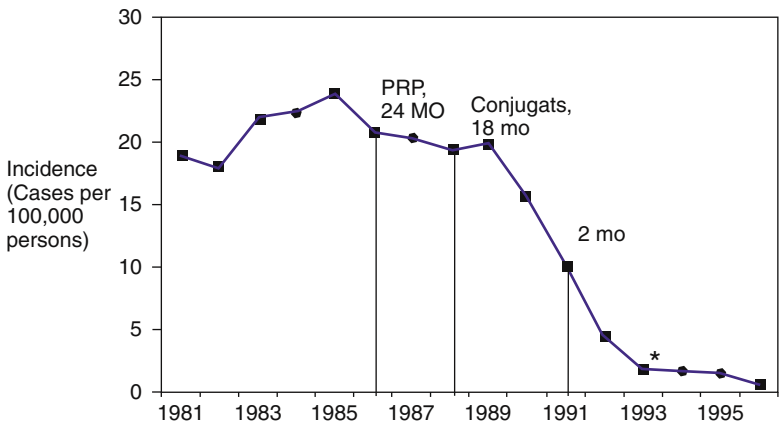


Figure 3. Hib meningitis in children less than 5 years old according to the National Bacterial Meningitis Reporting System, 1980–1991. Licensure dates and recommended ages of administration of PRP and conjugate vaccines are indicated (http://www.hhs.gov/nvpo/concepts/intro6.htm). * Active laboratory-based surveillance started, increasing sensitivity.

Table 3. *H. influenzae* Infections for Active Bacterial Core Surveillance Areas, 2006

Age (years)	Hib, # (rate)	Non-b, # (rate)	Non-typeable, # (rate)	Unknown type, # (rate)
< 1	2 (0.42/100,000)	10 (201/100,000)	31 (6.5/100,000)	4 (0.83/100,000)
1	0 (0.0)	4 (0.83/100,000)	3 (0.62/100,000)	1 (0.21/100,000)
2–4	3 (0.21/100,000)	5 (0.35/100,000)	12 (0.84/100,000)	1 (0.07/100,000)
5–17	1 (0.02/100,000)	3 (0.05/100,000)	13 (0.21/100,000)	1 (0.02/100,000)
18–34	0 (0.0)	6 (0.07/100,000)	22 (0.09/100,000)	7 (0.09/100,000)
35–49	1 (0.01/100,000)	15 (0.18/100,000)	37 (0.45/100,000)	11 (0.13/100,000)
50–64	4 (0.07/100,000)	29 (0.48/100,000)	53 (0.87/100,000)	15 (0.25/100,000)
>64	5 (0.12/100,000)	60 (1.5/100,000)	164 (04.1/100,000)	30 (0.75/100,000)

rates, in low socioeconomic status, in host susceptibility, or in high levels of Hib carriage in the population. The low level of Hib disease seen among children after the introduction of the Hib vaccine depends on maintenance of high post-immunization antibody titers among the age groups at greatest risk of invasive infections (those younger than age 5 years); failure to do so has resulted in re-emergence of increased rates of infections in the United Kingdom[115] and in Alaska (see Vaccine failures in Section 9.1.1). Among patients ≥6 months of age with Hib infection identified in the ABC surveillance program, 19 (17%) had unknown vaccination status, 31 (28%) were unvaccinated, 22 (20%) were undervaccinated, and 39 (35%) had completed the primary series.[116]

The mortality rate among 197 Hib cases reported in 1998–2000 through the ABC surveillance program[116] was 8%. Of these, 11 (79%) patients <6 months of age were unvaccinated and 3 (21%) patients >6 months of age were undervaccinated. Also similar to the pre-vaccine era, the most common types of Hib infection among children are bacteremia and meningitis.

In spite of high vaccine coverage in most of the United States, outbreaks of Hib disease still occur among poorly vaccinated populations. In Pennsylvania between December

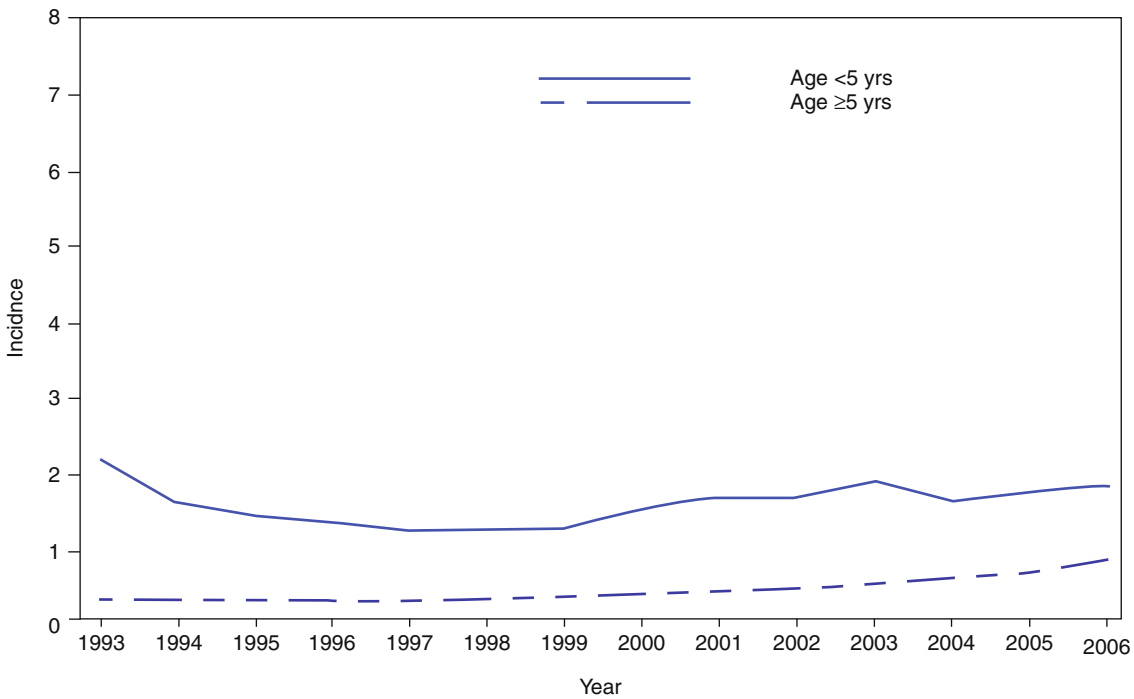


Figure 4. *Haemophilus influenzae* invasive disease incidence* by age group, United States, 1992–2005 as reported to the National Notifiable Diseases Surveillance System. MMWR March 30, 2007.
* Per 100,000 population.

Table 4. Incidence of Hib Infections in US Children < age 4 Years by Race and Ethnicity, 1994–1995

Race/ethnicity	Hib, # (rate)	Unknown type, # (rate)
Non-Hispanic		
White	102 (0.40/100,000)	150 (0.59/100,000)
Black	32 (0.55/100,000)	52 (0.89/100,000)
American Indian	10 (2.83/100,000)	3 (0.84/100,000)
Asian or Pacific Islander	3 (0.20/100,000)	6 (0.40/100,000)
Hispanic	7 (0.11/100,000)	31 (0.49/100,000)

1999 and February 2000, eight cases of Hib invasive infection occurred in unvaccinated children <5 years old and six from communities with lower Hib vaccination coverage and higher Hib carriage.[117] One objective of the Healthy People 2010 initiative of the Centers for Disease Control (http://www.healthypeople.gov) is to reduce the rate of type b disease in children <5 years of age to 0/100,000 through widespread use of the Hib vaccine.

5.1.3. Current Hib Epidemiology, US Adults. Among adults, the incidence rates are highest among those aged ≥65 years (Table 3),[118] most likely reflecting increased susceptibility due to immune senescence[119] and increased co-morbidities among older Americans that lead to increased susceptibility to bacterial infections in general. A 1996–2004 study from Illinois demonstrated that 56.6% of Hib infections occurred in adults, 16% among individuals 50–64 years old, and 27.6% among individuals ≥65 years old.[118] The relationship between childhood Hib vaccination and Hib disease rates in adults is underscored by the observation in the United Kingdom that increased rates of infection among children due to vaccine failure was associated with a subsequent increase in disease rates in adults.[120]

5.1.4. Hib Epidemiology Worldwide. Globally, Hib is estimated to cause 2–3 million cases of serious disease, notably pneumonia and meningitis, and 450,000 deaths in young children each year.[121] Most countries in the Americas, Western Europe, and Australia have high Hib vaccine coverage and low incidence of infection. Vaccine has not yet been introduced in most of Africa, the Middle East, the Far East, Eastern Europe, and Russia and the former Soviet Union countries. In these countries, Hib remains a major bacterial pathogen responsible for endemic meningitis.[122] The actual burden of disease in the Middle East and North Africa is difficult to assess, as surveillance systems are weak, data collection is sporadic, and bacteriologic confirmation of the etiologic agent in invasive infections is infrequent.

5.1.5. Risk Factors for Hib Infections. Among vaccinated populations, risk factors in children for invasive *H. influenzae* type b infections include failure to receive the complete Hib vaccine series, underlying immunodeficiency, sickle cell disease, and HIV-AIDS.[123] In non-vaccinated populations, risk factors include young age (ages 2 months to 5 years) and exposure to a person infected with Hib, such as occurs among households and in day care centers. Genetic variations in host immune responses to polysaccharide capsules may play a role in the development of an effective immune response to Hib and, thus, in altered susceptibility to infection.[124] In addition, preceding viral respiratory tract infections and episodes of acute otitis media are associated with the development of invasive Hib infections. Furthermore, environment factors that increase exposure of young children to Hib appear to contribute to increased infection, such as household crowding and presence of siblings.[112] In multiple studies, day care center attendance has been shown to be an independent risk factor.[125] Conversely, breast feeding has been shown to be protective against Hib infection.[107]

5.2. Epidemiology of Infections Caused by Types a, c–f *H. influenzae*

H. influenzae possessing non-b capsules, e.g., types a and c–f, cause invasive infections but at much lower rates than type b strains. Infection rates range from 0.05 to 2.1/100,000, with the highest rates occurring among infants <1 year of age based on data from the ABC surveillance program.[126] Among the five non-b capsular types, the majority of invasive infections are caused by types f (58%) and e (28%).[118]

A relatively high incidence of type a invasive infections has been reported among indigenous populations of North America. Since the advent of the type b vaccine, type a strains are now the most common serotypes seen among invasive *H. influenzae* infections, representing 24% of Alaskan strains and 89% of Northern Canadian strains.[127] Among Navajo and White Mountain Apache children <5 years old, from 1988 to 2003, the annual rate of type a infections in this population was 20.2/100,000 and types c, d, and f infections 4/100,000, with the predominant diseases being meningitis (50%) or pneumonia (27.6%).[42] Increased rates of a, e, and f invasive infections have not occurred with widespread use of the Hib vaccine (which has no effect in preventing infections with non-b strains); infections with types c and d occur too infrequently for a meaningful assessment of changing trends.[42,128] Thus, "sero-type replacement," such as has been seen with *S. pneumoniae* infections after introduction of the seven valent conjugate *S. pneumoniae* vaccine, apparently has not occurred following introduction of the Hib vaccines.

Pulsed-field gel electrophoresis (PFGE) analysis of *H. influenzae* type a isolated from 32 Navajo with invasive

infection showed them to be 90% similar using digestion with the restriction enzyme *Bln* I and 91% similar using digestion with *Sma* I. Control type a strains isolated from seven US or Canadian children showed 63 and 36% similarity, respectively,[42] suggesting limited genetic diversity of strains isolated on the Navajo reservation, in spite of the fact that they were isolated from geographically and temporally distinct cases.

Invasive infections in children with type a *H. influenzae* can be very severe, similar to those caused by type b strains. Genomic analysis of type a strains showed duplication of the *cap* region, similar to that seen in Division I Hib strains.[129] Furthermore, several had the same partial deletion of one of the *bexA* genes as seen in type b strains, which is thought to stabilize the duplication and result in increased capsule production and virulence.[84] Examination of other non-b, encapsulated *H. influenzae* revealed single copies of the *cap* region in types c and d strains, but types e and f strains contained duplicated copies, some with the *bexA* deletion in one copy.[130]

5.3. Epidemiology of Infections Caused by Non-typeable *H. influenzae*

The rate of NTHi invasive infections among all age groups, as identified in the Active Bacterial Core surveillance (ABCs) network, is 0.95/100,000 individuals. The highest rates are in children <1 year of age and adults >65 years (Table 3). NTHi sepsis among newborns, which is estimated to occur in 1.6–1.9/100,000 live births,[131] is associated with maternal peripartum infections[132] and infant prematurity.[133] Among adults, most NTHi invasive infections are associated with a variety of acute and chronic medical conditions.[134,135] Since the Hib vaccine protects only against infections caused by type b organisms, this vaccine has no impact on the epidemiology of non-typeable *H. influenzae* infections.

Systematic surveys of non-invasive non-typeable *H. influenzae* infections are not available but have been reported in the context of clinical studies. Eskola et al.[136] reported rates of *H. influenzae* acute otitis media to be 203 episodes/1000 children/year among healthy Swedish children under 18 months of age. Interestingly, these rates were lower than those of healthy children who received the seven-valent *S. pneumoniae* conjugated vaccine (283 episodes/1000 children/year), suggesting that altered pharyngeal flora following *S. pneumoniae* conjugated vaccine resulted in increased *H. influenzae* otitis media.

Occasional outbreaks of non-typeable *H. influenzae* have been reported, primarily associated with nursing homes[137,138] or respiratory rehabilitation units.[139,140]

6. Mechanisms and Routes of Transmission

Because *H. influenzae* live exclusively in humans, person-to-person transmission is vital to survival of these bacteria. The organisms are spread via infected respiratory droplets and establish colonization in the upper respiratory tract (the naso- and posterior oro-pharynges). Murphy et al.[141] demonstrated that Hib in nasal secretions were able to survive up to 18 h on tissue paper, wax paper, and dry gauze. Hib could not, however, be isolated from swab cultures of fomites in day care settings known to be exposed to children carrying Hib. Hib is recovered in higher titers and for longer duration from laminated or steel surfaces than from porous surfaces such as paper or cloth and the recovery is improved with higher inocula.[142] To reduce the potential for transmission of Hib among hospitalized patients, respiratory isolation precautions are recommended for patients with *H. influenzae* type b infections for 24 h after initiating appropriate antibiotic therapy.

Molecular epidemiologic tools (see Section 3.2.2) allow tracking the transmission of *H. influenzae*. Strains isolated from family members show high genetic similarity, suggesting intrafamilial spread.[38,143,144] A single genotype of non-typeable *H. influenzae* may be found in multiple children attending a day care center, demonstrating transmission within day care.[34,35,145]

Environmental factors directly related to transmission of *H. influenzae* in humans are difficult to identify, but factors associated with sharing of genotypically identical NTHi in day care centers may serve as a marker of transmission. Such factors include a higher number of children in the day care center, suboptimum hygiene habits, and pacifier use.[146]

Bacterial factors may also be important in person-to-person spread of *H. influenzae*, but have not been clearly identified. Transmission factor candidates include absence of adhesins or adhesin variants that lose the ability to adhere to certain epithelial cells.

7. Pathogenesis and Immunity

7.1. *H. influenzae* Colonization

With rare exceptions, *H. influenzae* exist exclusively in the upper respiratory tracts of humans where they live as asymptomatic commensals. As such, colonization of the host's pharynx is the first step in the pathogenesis of any *H. influenzae* infection.

7.1.1. Colonization with *H. influenzae* Type b (Hib). Pharyngeal colonization with type b organisms in non-immune individuals is relatively rare, with point prevalence rates ranging from 0.7 to 5.3%.[147] Cumulative (over

18 months) prevalence rates of Hib carriage among children in day care, however, were between 48 and 71% in toddlers and preschool age children, with lower rates among infants and adults and school age children,[148] demonstrating that over time, many unimmunized children may be colonized with Hib. Introduction of conjugate Hib vaccines has further reduced carriage of Hib in young children[149]; the appearance of a herd effect among vaccinated populations suggests that reduced colonization rates extend beyond those children who are immunized.[113]

7.1.2. Colonization with Non-typeable *H. influenzae* (NTHi). Asymptomatic colonization with non-typeable *H. influenzae* in healthy children is extremely common, with point prevalence rates of approximately 25 to 77–84%[34,150–154]; the wide variability may be related to differences in the populations studied and in culturing techniques.[35] Colonization with non-typeable *H. influenzae* begins early in life, increases in frequency during childhood, and then decreases during adolescence and adulthood.[155,156]

Recent studies have demonstrated that NTHi colonization, as determined by bacterial culture of respiratory samples, is a very dynamic process, characterized by rapid bacterial turnover[152,157,158] and carriage of multiple NTHi strains at any one time.[150,159–162] Multiple *H. influenzae* genotypes are frequently present in the upper respiratory tracts of adults with cystic fibrosis,[92] of adults with chronic obstructive pulmonary disease,[161] and of children in day care centers.[34,35] Studies from healthy children attending day care demonstrated a 62% turnover rate of *H. influenzae* isolates from week to week, and 37–43% of throat cultures contained two or more genetically distinct NTHi (range = 0–5).[34,35] A study of chronic carriage in four healthy adults over 7 months showed carriage with multiple genotypes over time, co-colonization with several genotypes at a single point in time, intermittent recovery of individual genotypes, and prolonged carriage (up to at least 198 days) with a single genotype.[36] The best explanation for this dynamic pattern of colonization is the presence of a large population of organisms colonizing the respiratory tract, with ongoing evolution of the organisms and acquisition and loss of specific genotypes.

Risk factors associated with asymptomatic NTHi colonization include exposure to tobacco smoke[146,151,163] and attending day care.[145] In the day care setting, risk factors include ≥5 classrooms in the center and suboptimal hygiene practices such as minimal handwashing by staff after eating and by children after wiping their own noses.[146]

7.1.3. *H. influenzae* and Biofilms. Growing evidence confirms that *H. influenzae* inhabit mucosal surfaces as biofilms[164,165] and several *H. influenzae* bacterial factors have been shown to be important in biofilm formation, including presence of hemagglutinating pili[166] and type IV PilA,[167] and sialylation of lipooligosaccharides.[168] Bacteria growing in a biofilm are in a different metabolic state than those grown under planktonic conditions and thus exhibit different phenotypic characteristics; for example, the expression of lipooligosaccharide is altered during NTHi growth in a biofilm.[166] The observation that biofilm growth increases the phosphorylcholine content and decreases the potency of non-typeable *H. influenzae* endotoxins suggests a mechanism for reduced mucosal inflammation during colonization compared to infection.[169]

7.2. *H. influenzae* Virulence Factors

While *H. influenzae* asymptomatically colonize the human pharynx, they cause infection and disease at other body sites. These bacteria, like other pathogens, possess a number of factors that facilitate its pathogenicity in humans, such as factors that allow the bacteria to adhere to mucosal surfaces and promote colonization, that evade the host's immune system, that degrade host protective factors, and that stimulate an inflammatory response in the host that then causes the symptoms of the disease.

7.2.1. Polysaccharide Capsule. The most important virulence factor associated with pathogenic *H. influenzae* is the type b capsule, which is carried by the majority of *H. influenzae* strains that cause invasive infections such as bacteremia, meningitis, septic arthritis, cellulitis, and epiglottitis. Strains carrying types a and c–f capsules also cause invasive infections, but their incidence is much lower than that of type b infections in non-immune populations. The type b capsule protects the bacteria from phagocytosis by interfering with the binding of complement and opsonizing antibodies to the bacterial surface.[170] In addition, the strong negative charge of the polysaccharide capsule may provide electrostatic repulsion to phagocytic cells.[171]

7.2.2. Attachment Factors. *H. influenzae* possess a number of adhesins that facilitate bacterial adherence to epithelial cells and the respiratory mucosa and, thus, facilitate colonization, the initial event in the pathogenesis of *H. influenzae* infection and disease.

Hemagglutinating pili are complex, filamentous structures that extend from the bacterial surface and are antigenically very diverse.[172,173] Expression of pili is phase variable and expression occurs more commonly on bacteria isolated from mucosal surfaces than from sites of invasive infection. Adherence is mediated through the HifE proteins, which are located at the tips of the pili[174] and appear to bind to sialic acid-containing lactosylceramides on cell surfaces.[175]

High molecular weight adhesins (HMW) are present only in non-typeable *H. influenzae*. These proteins show high sequence diversity and bacteria that express certain allelic forms have different binding affinities for transformed epithelial cells.[94] HMW expression is also phase variable[88] and variants lacking the *hmw* genes are more prevalent among strains isolated from throat specimens than from middle ear specimens, suggesting the role of HMW in the pathogenesis of acute otitis media.[176,177]

Hia is a surface adhesin found primarily on non-typeable strains that do not express HMW. The protein is homologous with Hsf, also known as fibrils, found on type b strains.[178]

The serine protease Hap, another surface adhesin, is structurally but not functionally related to IgA protease and undergoes autoproteolysis with release of the adhesive region, Hap_s. The protein self-assembles into fibrillar structures that bind to each other, facilitating the formation of bacterial microcolonies.[179] In addition, host-mediated inhibition of the autoproteolysis results in retention of the central protease region on the bacterial cell surface, which facilitates adherence of the bacteria to respiratory epithelial cells.

P5 (also known as P5 fimbrin) are thin fibillae-like structures that mediate adherence to the carcinoembryonic antigen family of surface proteins on respiratory cells.[180] P5 proteins have been demonstrated to exhibit antigenic drift during the process of human colonization with *H. influenzae*.[181]

Several additional adhesins have been described from *H. influenzae* and include lipooligosaccharides,[182] the opacity-associated protein OapA[183] and the type IV pilus adhesin PilA.[184]

7.2.3. Lipooligosaccharide (LOS). *H. influenzae* lipopolysaccharides lack the O-specific side chain found in enteric gram-negative bacteria and, thus, are termed by many investigators lipooligosaccharides (LOS). They have been shown to be important determinants of virulence in animal models of bacteremia.[185] The core LOS structure is a short oligosaccharide composed of three conserved heptoses, which may be modified with a variety of carbohydrate moieties (such as glucose, galactose, or heptulose) and non-carbohydrate moieties (such as phosphoethanolamine, phosphocholine, sialic acid, and glycine).[186] Many of the glycosyl transferases responsible for LOS modifications are phase variable, contributing to the widely variable structure of *H. influenzae* LOS.[187]

One LOS modification, the addition of phosphatidylcholine (ChoP), has been associated with acute otitis media in the chinchilla model.[188] While strains expressing ChoP are commonly found among pharyngeal commensal strains,[189] most invasive strains do not express this LOS modification,[190] suggesting its role in colonization and not in disease. This suggestion is corroborated by the observation that ChoP expressing *H. influenzae* are more susceptible to C-reactive protein-mediated serum killing.[189]

Another LOS modification, the addition of sialic acid, also confers serum resistance to *H. influenzae*.[191] Sialic acid is a common surface constituent of human cells and its presence on *H. influenzae* may protect the bacteria from the host immune system through molecular mimicry. Additional modifications involving the glycosyl moieties and O-acetylation of LOS result in increased serum resistance and increased virulence of *H. influenzae*.[186]

H. influenzae LOS appears to stimulate an innate immune response in the human host through interactions with Toll-like receptor 4 (TLR4),[192] presumably thus activating the NF-κB pathway. As a result, a variety of inflammatory molecules are produced, including IL-6, IL-8, TNFα, and MCP-1.[193]

7.2.4. Iron and Heme Uptake. Because *H. influenzae* has an absolute growth requirement for iron, as supplied by exogenous heme, the organism has several, possibly redundant and/or complementary, mechanisms for the acquisition of iron from heme and related iron-containing compounds. Hgp proteins are encoded by a group of four (*hgpA-D*) phase-variable genes and are involved in the utilization of heme from both hemoglobin and hemoglobin:haptoglobin complexes. Different strains of NTHi contain between one and four *hgp* genes. Mutant NTHi strains lacking all *hgp* genes showed reduced virulence in a chinchilla model of otitis media as compared to wild type[194] as well as reduced ability to initiate and sustain type b bacteremia in weanling rats.[195] The *hux* operon, consisting of three genes, encodes proteins important in binding heme and heme:hemopexin. A mutant Hib strain lacking *hxuA-C* produced lower bacterial titers during bacteremia, with associated improved survival rates, in 5-day-old rats as compared with the wild-type strain.[196]

Other iron metabolism factors, such as the heme-binding lipoprotein HbpA, transferrin binding proteins Tbp1 and Tbp2, and the hemin receptor HemR deserve further study as potential virulence factors of *H. influenzae*. Epidemiology-based prevalence studies of potential Hi virulence factors have shown that *hgpB* (encoding hemoglobin binding protein) and *hemR* (encoding hemin receptor) are present in 100% of *H. influenzae* isolated from infections in the middle ear and only 70 and 83%, respectively, in commensal strains isolated from the throats of healthy children[177] suggesting their potential role in acute otitis media.

7.2.5. IgA Protease. IgA protease is an endopeptidase that inactivates the immunoglobulin IgA1 by enzymatic

cleavage. Although all *H. influenzae* strains possess *iga*, the gene has high sequence variability between strains. Nontypeable *H. influenzae* isolated from infected patients have significantly higher levels of IgA1 protease activity than strains isolated from asymptomatic carriers.[197]

7.2.6. Other Virulence Factors. Additional virulence factors of *H. influenzae* include protein D, a highly conserved lipoprotein that compromises the mucociliary escalator in respiratory cells in tissue culture[198] and has been shown to induce otitis media in a rat model.[199] Furthermore, Sap proteins, which confer resistance to antimicrobial peptides in the respiratory tract, contribute to acute otitis media in chinchillas.[200] LuxS, involved in the quorem-sensing activity of gram-negative bacteria, has also been shown to modulate acute otitis media in chinchillas.[201]

7.3. Immunity

The relationship between immunity, as defined by the ability of blood samples taken at various ages to kill *H. influenzae* type b bacteria in vitro, and protection against infection with *H. influenzae* type b has been recognized since the landmark studies of Fothergill and Wright in 1933,[4] Figure 5. Subsequently, the major component of the protective factor in blood has been shown to be antibodies against the type b capsule. As a result of these studies, vaccines, composed first of the purified type b capsule and later of the purified capsule conjugated to a carrier protein, have been developed and have nearly eliminated type b disease in populations with high vaccine use (see Section 9.1.1, Type b *H. influenzae* vaccines). The success of vaccines containing the purified capsule of *H. influenzae* underscores the important role of immunity to the type b capsule in protecting against disease. Immunity against a variety of additional *H. influenzae* surface structures, particularly those of nontypeable strains, has also been well studied in work directed at developing additional *H. influenzae* vaccines (see Section 9.2, Vaccines against NTHi).

7.3.1. Immunity Against the Type b Capsule. The type b capsule of *H. influenzae* is a complex carbohydrate composed of repeating units of polyribosyl ribitol phosphate or PRP. Natural immunity against *H. influenzae* type b develops in healthy humans during early childhood and is the result of natural antibodies directed against the type b capsule, among other possible antigens. The antigenic stimuli for the development of natural anticapsular antibodies is unclear, but may be related to asymptomatic pharyngeal colonization with type b *H. influenzae* or colonization with bacteria that possess cross-reacting capsular material, such as *E. coli* K100, *S. aureus*, *Bacillus pumilus*, *Lactobacillus plantarum*, or *Streptococcus faecium*.[202,203]

7.3.1.1. Age-Related Capsular Immunity. Relatively high levels of antibodies directed against the capsule of *H. influenzae* are present at birth because of transplacental transfer of maternal antibody to the fetus. These antibodies are degraded shortly after birth and fall to undetectable levels by age 6 months. Subsequently, the levels rise again as natural antibodies are generated and adult levels are achieved in most children by age 5 years.

Many young children under age 2 years are unable to generate an antibody response to purified capsular polysaccharides, including the PRP of *H. influenzae* even following systemic infection with Hib.[204] With increasing age and maturation of their immune systems, many children over age 2 years generate a brisk antibody response to antigenic stimulation with the type b capsule, either through infection with *H. influenzae* type b or through vaccination with purified PRP.

7.3.1.2. Role of T Cells in Capsular Immunity. Failure of young children to illicit a protective and enduring antibody response to purified type b capsule antigen is a function of the immaturity of their immune systems and their limited ability to activate T-helper cells upon exposure to purified polysaccharide antigens. T-helper cells are important in the maturation, differentiation, and proliferation of B cells that produce specific antibody. Thus, young children under age 2 years illicit a T-independent (i.e., thymus independent) response to purified polysaccharides, which is characterized by low levels of antibody, primarily IgM antibody with little IgG antibody, and poor immunologic memory that results in a short-lived, non-boostable immune response. On the other hand, most children over age 2 years are able to illicit a T-cell-dependent response to purified polysaccharide antigens in which activated T cells provide the appropriate immune signals to stimulate activation of B cells, resulting in the production of antibody that is long lasting and demonstrates an anamnestic response upon re-exposure, thus immune memory.[205]

To convert T-cell-independent *H. influenzae* capsular polysaccharides to T-cell-dependent antigens, the purified PRP capsule has been chemically conjugated to several different protein carriers, including diphtheria toxoid; CRM_{197}, a nontoxic derivative of diphtheria toxin; OMP, an outer membrane protein of group B *N. meningitidis*; and tetanus toxoid (see Section 9.1.1, *H. influenzae* type b vaccines). The carrier proteins permit polysaccharide interactions with T cells to provide appropriate immune signals necessary to generate a T-cell-dependent response that stimulates B-cell proliferation, differentiation, and antibody production. These conjugated PRP antigens stimulate high levels of anticapsular antibodies in children as young as 2 months of age that persist for extended periods of time and show an anamnestic (memory) response upon boosting.

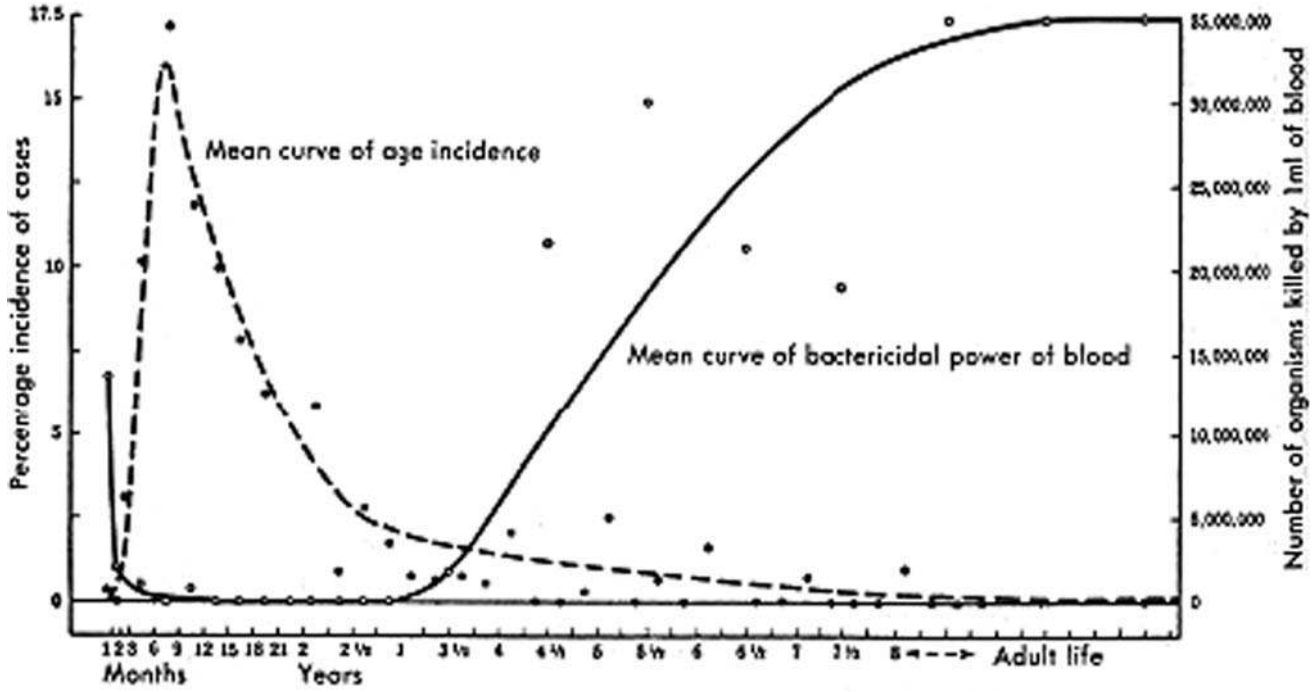

Mean curve of age incidence
Mean curve of bactericidal power of blood
Percentage incidence of cases
Number of organisms killed by 1ml of blood
Months
Years
Adult life

Figure 5. The relationship of age of *H. influenzae* type b meningitis to the bactericidal power of human blood against Hib at various ages.[4]

7.3.1.3. Biologic Activities of Capsular Antibodies. Antibodies against the type b capsule provide host protection against infection through several different mechanisms, including activation of complement, complement-mediated opsonophagocytosis, and direct, complement-mediated bacterial lysis.[206] Serological correlates of protection for type b antibodies have been estimated to be 0.15 μg/ml of anti-PRP antibody based on protective levels in adult sera[207,208] and 1 μg/ml of anti-PRP antibody based on post-vaccination levels that offered protection for a year[209] (see Section 9.1.1.2, Serologic correlates of immunity).

7.3.1.4. Mucosal Immunity. The role of mucosal antibodies in protection against *H. influenzae* infection remains unclear. Secretory IgA anticapsular antibodies are present in mucosal secretions of children following *H. influenzae* infection or vaccination with the Hib polysaccharide vaccine.[210–212] IgA antibodies, however, do not bind complement and, thus, are thought to lack bactericidal activity against *H. influenzae*. Their role, rather than bacterial killing, may be to impair adherence of *H. influenzae* to respiratory tissues or to facilitate bacterial clumping with resultant increased clearance from the respiratory tract.

Although purified polysaccharide Hib vaccines appear to stimulate antibodies in respiratory secretions, either IgA or serum-derived IgG antibodies that are present in the secretions through transudation, immunity stimulated by these carbohydrate antigens does not reduce colonization of *H. influenzae* type b. On the other hand, the protein conjugated polysaccharides, including conjugated *H. influenzae* type b vaccines, stimulate antibodies that significantly reduce Hib colonization.[213]

In addition to adaptive, antibody-derived immunity, the innate immune system, which includes antimicrobial peptides present in the mucosa that lines the respiratory tract, also acts against respiratory pathogens. The cationic peptides β defensins[214] and the human cathelicidin LL37[215] as well as lysozyme[216] appear to bind to various components of *H. influenzae* lipooligosaccharides, resulting in bacterial cell death.

7.3.2. Immunity Against Non-capsular Antigens. The success of the Hib vaccines in reducing *H. influenzae* type b disease clearly emphasizes the importance of capsular antibodies in providing protective immunity against *H. influenzae* type b. The importance of immunity against non-capsular antigens in providing immune protection against *H. influenzae* disease is less clear. Many surface proteins stimulate antibodies in humans[217] that exhibit bactericidal and/or opsonophagocytic activities but their role in protection against disease is difficult to discern, in part because of significant antigenic variation among many of the surface proteins.[89] The use of the chinchilla model of *H. influenzae* has been valuable to define the ability of

non-capsule antigens to stimulate immune protection, which is often partial protection, to prevent acute otitis media.[218]

8. Patterns of Host Response

8.1. Infections with Typeable *H. influenzae*

8.1.1. Diseases Caused by Encapsulated *H. influenzae*. Prior to the introduction of the type b vaccine, type b *H. influenzae* was the most common cause of bacterial meningitis in children in the United States. In addition, type b strains commonly caused epiglottitis, bacteremia, septic arthritis, periorbital and facial cellulitis, and pneumonia. Less common manifestations of invasive type b *H. influenzae* include osteomyelitis, pericarditis, endocarditis, epididymitis, endophthalmitis and peritonitis. *H. influenzae* expressing the other capsular types, a, c, d, e, and f, also cause occasional invasive infections such as bacteremia and meningitis.

Bacteremia with encapsulated *H. influenzae* almost always precedes *H. influenzae* infections at distal sites and is the means by which the bacteria access meninges, joints, pericardium, and other tissues. In non-immune children, Hib bacteremia without focus is a relatively common illness, which presents with fever and no localizing symptoms of infection. While *H. influenzae* bacteremia without focus may spontaneously resolve with no residual clinical sequelae, it may result in Hib seeding of distant tissues, commonly the meninges.

Bacterial meningitis, including that caused by encapsulated *H. influenzae*, is an extremely serious, acute infection of the membranes surrounding the brain and spinal cord. Early symptoms include fever, lethargy, and irritability and later symptoms include vomiting, stiff neck, and seizures. Children with symptoms suggestive of bacterial meningitis require immediate medical care to diagnose and appropriately treat this infection. Mortality rates approximate 5–10% and morbidity includes mental retardation, blindness, hearing loss, and persistent seizures.

In non-immune individuals, *H. influenzae* type b is the predominant cause of epiglottitis (infection of the epiglottis) which presents with fever and rapidly progressing, severe respiratory distress from upper airway obstruction. Hib is also a significant cause of septic arthritis, which presents with fever and joint swelling accompanied by redness of the overlying skin. Facial cellulitis is an infection of infants that is nearly always caused by *H. influenzae* type b and is characterized by fever and rapidly developing swelling of the skin of the face with redness that progresses to purple discoloration.

All cases of *H. influenzae* invasive disease, including type b and non-type b, should be reported to the Centers for Disease Control and Prevention through the appropriate local or state public health departments.

8.1.2. Mechanisms of Disease Caused by Encapsulated *H. influenzae*. Type b, and to a lesser extent non-b encapsulated *H. influenzae*, gain access to the blood stream by unknown mechanisms, circulate, and seed various organs, such as meninges, skin, pericardial or pleural spaces, and joints. Type b strains are thought to directly seed the epiglottis, and possibly the lower respiratory tract, from the pharynx. All forms of infection with encapsulated *H. influenzae* are associated with bacteremia, either as a primary or a secondary event.

The most important virulence factor for invasive *H. influenzae* disease is the type b capsule, which differs chemically and antigenically from the capsules of the other encapsulated *H. influenzae*. The mechanisms of type b capsule-mediated *H. influenzae* virulence include resistance to phagocytosis because of the strong negative charge of the capsule and resistance to complement-mediated bacterial lysis. In most type b strains, the capsule in encoded by a duplicated *cap* gene region with a 1.2 kb deletion in one of the *bexA* genes.[219] Up to five copies of the *cap* region has been described in invasive Hib strains and increased copy number has been correlated with increased capsule elaboration[220] and with decreased susceptibility of the bacteria to both complement-mediated lysis and opsonization.[221] Invasive strains of type a *H. influenzae* have also shown the partial *bexA* deletion, suggesting an explanation for their virulence.[222]

While the presence of the type b capsule allows *H. influenzae* to survive during bacteremia, it is the host inflammatory response to the infection that is associated with the manifestations of the disease. Bacterial factors other than capsule, for example lipooligosaccharides, stimulate inflammatory cytokines such as Il1β and TNFα[223,224] that lead to the symptoms associated with infection.

8.2. Infections with Non-typeable *H. influenzae* (NTHi)

H. influenzae that lack capsular polysaccharides are phylogenetically unique from encapsulated *H. influenzae*,[23] with different outer membrane protein patterns.[225] While NTHi strains are highly diverse, well-defined phylogenetic groups that differ in gene content have been identified using MLST but these groups do not correlate with NTHi diseases.[226]

8.2.1. Diseases Caused by NTHi. The majority of infections caused by non-typeable *H. influenzae*, which lack the polysaccharide capsule, in healthy individuals are limited

to the respiratory tract and include acute otitis media and sinusitis in children and pneumonia and bronchitis in adults. Since the introduction of the conjugated *S. pneumoniae* vaccine in children, NTHi has emerged as the major cause of acute otitis media, responsible for between 30 and 52% of these infections.[136] Furthermore, NTHi cause approximately a third of episodes of acute sinusitis.[227]

Community-acquired pneumonia, particularly in children in developing countries and among the elderly, is often caused by NTHI.[228,229] Non-typeable *H. influenzae* are the most commonly seen bacteria in acute exacerbations of chronic obstructive pulmonary disease (COPD)[230,231] and appear to contribute to COPD progression.[232] Acute exacerbation in COPD patients often occurs with acquisition of a new strain of *H. influenzae*, relative risk = nearly 3.0.[230]

In addition to persons with underlying bronchial and pulmonary chronic illnesses, patients with anatomic defects such as primary ciliary dyskinesia or patients with common variable immunodeficiency, IgA deficiency or IgG3 deficiency may have bronchitis or pneumonia caused by NTHi. Furthermore, non-typeable strains may rarely cause bacteremia and/or meningitis, particularly among individuals with underlying medical conditions such as cerebral spinal fluid leaks, prematurity, lymphoproliferative syndromes, or hypogammaglobulinemia.[233,234]

8.2.2. Mechanisms of Disease Caused by NTHi. The initial interaction of NTHi with a human host is adherence to the respiratory mucosal cells, thus establishing asymptomatic colonization of the pharynx. NTHi may, subsequently, gain access to the middle ear space from the pharynx by ascent through the Eustachian tube and subsequently initiate an inflammatory response resulting in acute otitis media.[235] In addition NTHi presumably gain access to the respiratory sinuses through the ostia that naturally provide egress of sinus secretions into the pharynx and, once established in the sinuses, cause acute sinusitis. In patients with damaged airways, such as those with cystic fibrosis or COPD, the bacteria descend from the pharynx into the bronchi where they may establish chronic infection.

While NTHi lack toxins and a capsule, which is the major pathogenic factor of type b *H. influenzae*, they possess a number of potential virulence factors that may contribute to disease, including adhesins, lipooligosaccharide, IgA protease, and other outer membrane proteins.[91] Interactions between NTHi and host cells through the Toll-like receptors TLR2 and TLR4 as well as the platelet-activating factor receptor PAFR have been shown to initiate intracellular signals through the mitogen-activated kinase cascade or by way of p38 activation that are involved in many human inflammatory pathways.[235]

8.3. Infections Caused by Unique *H. influenzae* Strains

8.3.1. Conjunctival Infections. Bacterial conjunctivitis (presenting with mucoid or purulent discharge from the eyes[236]) is often caused by non-typeable *H. influenzae*, occurring up to 40% in some series[237] with even higher occurrence in children.[238] The *H. influenzae* isolated from the eye exudates of these patients are genetically unique and appear to represent the bacterium originally observed by Koch[239] in pus from the eyes of patients with conjunctivitis in Egypt and cultured from cases of conjunctivitis in the United States by Weeks,[240] hence the term "Koch–Weeks bacillus." Originally designated *Haemophilus aegyptius*, these strains are very closely related to *H. influenzae*.[226,241] Several investigators suggest they do not belong to a separate species based on DNA hybridization studies[242] and on the observation that no one single feature differentiates the two organisms.[243,244] Thus, acute conjunctivitis isolates are often designated "*H. influenzae*, biogroup aegyptius."

Simultaneous presentation of conjunctivitis and acute otitis media, the so-called conjunctivitis–otitis syndrome, is strongly associated with isolation of *H. influenzae* from both sites. Pulsed field gel electrophoresis analysis of paired *H. influenzae* isolates from the conjunctival exudate and middle ear fluid of patients showed identical strains in each patient.[245]

8.3.2. Brazilian Purpuric Fever. In late 1994, 10 children in the town of Promissao in Sao Paulo State, Brazil, developed a severe illness characterized by high fever, abdominal pain and vomiting, hemorrhagic skin lesions, vascular collapse, and death. In the majority of affected children, the acute illness was preceded by an episode of acute conjunctivitis. This syndrome, called Brazilian purpuric fever, subsequently affected over a hundred children in Brazil and cultures of the blood or cerebrospinal fluid of several patients yielded non-typeable *H. influenzae* that were genetically related to *H. influenzae* biogroup aegyptius.[246]

The *H. Influenzae* strains associated with Brazilian purpuric fever (so-called BPF strains) are identical by a wide range of genotypic and phenotypic criteria[247] and resist killing by normal human sera.[248] In spite of extensive effort, no unique bacterial factor that underlies the profound virulence of this clone compared to other *H. influenzae* biogroup aegyptius strains has been identified.

8.3.3. Genital Infections. Although *H. influenzae* reside in the human respiratory tract and cause respiratory tract infections or occasionally bacteremia and invasive infections, a cryptic genospecies of non-typeable *H. influenzae* causes urogenital, puerperal, and neonatal infections.[249,250] These biotype IV strains have a unique outer membrane P6 protein[251] and are genotypically (as

assessed by MLEE) divergent from all other *H. influenzae* isolates.[23] 16S rRNA analysis has demonstrated that these genital strains are most closely related to *H. haemolyticus*, a species closely related to *H. influenzae* that has not been linked to human infection.[250]

9. Control and Prevention of *H. influenzae* Infections

9.1. Prevention of Type b Infections

9.1.1. Hib Vaccines. The polysaccharide of type b *H. influenzae*, polyribosyl ribitol phosphate (PRP), has many properties important as a vaccine antigen, including antigenic conservation among all type b strains, immunogenicity in humans, and induction of protection against Hib infection and disease. Thus PRP, licensed in 1985, was the first purified polysaccharide formulation used as an *H. influenzae* vaccine. Early studies of vaccines composed solely of the purified PRP demonstrated that children under age 2 years developed relatively low titers of serum antibodies that appeared insufficient to protect against invasive disease.[252] Antibody induced by purified PRP was found to wane quickly and immunization provided no immune memory.[253]

Based on the knowledge that polysaccharides conjugated to proteins stimulate enhanced immune responses and protective immunity in animals,[254] vaccines composed of purified PRP conjugated to a variety of protein carriers were developed,[255] Table 5.

The Hib conjugate vaccines differ in their immunogenicity, probably related to the nature of the carrier protein that acts as a hapten, and the method of coupling, either covalent linkage or chemical conjugation of the protein, to the polysaccharide. PRP-D is no longer used in young infants because of its poor immunogenicity and poor protection against Hib infection in Alaskan Native children, a population with a high level of natural disease.[256] PRT-T, PRP-OMP, and HBOC have all been shown to generate high levels of anti-PRP antibodies in infants and to be highly protective against Hib invasive infection.[257] Repeated administration of conjugate vaccines elicits booster responses, thus maximizing the level of the immune responses achieved.

9.1.1.1. Clinical Trials of Hib Vaccines. The large clinical trials designed to evaluate the efficacy of conjugate Hib vaccines used different strategies.[257] Classic double-blind placebo-controlled studies of PRP-D and PRP-OMP were conducted in Native American and Alaskan Native populations in which relatively small numbers of vaccine recipients were needed because of the high rates of Hib disease in the study populations. In contrast, PRP-D and HbOC were compared in trials in Finland, a population with relatively low levels of Hib disease, with estimates of efficacy based on historical data. A case–control study of HbOC was conducted in the United States in Los Angeles County, an area with relatively low coverage rates of the conjugated vaccine (PRP-D) licensed at that time.[258]

Because of the various study designs, comparing the efficacies of the vaccines is difficult. The only head-to head-comparison, PRP-D and HbOC, conducted in Finland with relatively low infection rates, showed no statistically significant differences in their efficacy.[259] The poor performance of PRP-D among Alaskan Natives is likely related to its poor immunogenicity, as trials of other conjugate vaccines in populations with high disease rates, PRP-T in Gambia[260] and PRP-OMP in Navajo Indians,[261] showed excellent vaccine efficacy.

9.1.1.2. Serologic Correlates of Immunity. The identification of levels of serum antibody that correlate with protection against vaccine-preventable infections is a keystone to understanding vaccine-induced immunity, as these correlates predict the efficacy of a vaccine against natural infection in the face of an average infecting dose of the bacteria.[262] Although mucosal and cellular immunity may have a role in protecting against *H. influenzae* infections, the presence of serum antibody directed at the type b capsule strongly correlates with protection. Several lines of evidence have defined the level of type b serum antibody that best correlates with protection. In an unvaccinated population, ≥ 0.15 μg/ml of antibody against the type b capsule correlated with the protection against disease,[252] which is consistent with protective

Table 5. Hib Conjugate Vaccines

Vaccine	Protein carrier	Trade name	Age groups*	Antibody response in infancy
PRP-D**	Diphtheria toxoid	ProHIBiT	15–59 months	Moderate after second dose
HbOC**	CRM197 mutant	HibTITER	2, 4, 6, and 12–15 months	Good after second dose
PRP-OMP	*N. meningitidis* outer membrane protein	PedvaxHIB	2, 4, and 12–15 months	Moderate after first dose
PRP-T	Tetanus toxoid	ActHIB	2, 4, 6, and 12–15 months	Good after second dose
PRP-T/DTaP	Tetanus toxoid	TriHIBit	Licensed for fourth dose only	Not applicable
PRP-OMP/Hepatitis B	*N. meningitidis* outer membrane protein	Recombivax	2, 4, 6, and 12–15 months	Moderate after first dose

* Licensed ages in the United States, **no longer available in the United States

values derived from gamma globulin prophylaxis and from declining maternal antibodies.[262] Among recent recipients of purified PRP Hib vaccine, ≥1 μm/ml antibody predicted long-term protection.[209] While these levels of protection are a useful generalization, they do not take into account the variations in IgG subclass and their distribution in human serum, the idiotype specificity of the antibodies, the antibody avidity, the functional capacity of various types of anticapsular antibodies, the type or intensity of bacterial exposure, or the specific techniques used to measure the antibodies.

9.1.1.3. Use of Current Vaccines. The dose of each Hib conjugate vaccine is 0.5 ml, given intramuscularly. In the United States, the recommended schedule is for all children to receive the primary vaccine series at ages 2 and 4 months for PRP-OMP or 2, 4, and 6 months for PRP-T, with a booster at age 12–15 months. Hib vaccines can be administered separately or in combination with other vaccines.[263] Monovalent Hib conjugate vaccines available in the United States are considered interchangeable for primary and booster immunizations. Two conjugated vaccines are available as combination vaccines, PRP-T/DTaP and PRP-OMP/hepatitis B. All Hib vaccines are safe, with only minor, local reactions occurring in some children. Vaccination with a specific Hib conjugate vaccine is contraindicated in persons known to have experienced anaphylaxis following a prior dose of that vaccine. Minor illnesses (e.g., mild upper-respiratory infection) are not contraindications to vaccination. Vaccination should be delayed in children with moderate or severe illnesses. In premature infants, the immunogenicity of *H. influenzae* type b conjugate vaccines varies widely among studies and may be affected by the type of carrier protein, by inclusion in a combination vaccine, and by an infant's overall health.[264]

Vaccine coverage rates in the United States for the primary series of Hib vaccine (three or four doses, depending on the product used for vaccination) are high, approximately 85%.[265] Use of a combination vaccine is associated with slightly higher coverage, odds ratio = 1.05.

9.1.1.4. Impact of Hib Vaccines. Widespread use of the Hib vaccine in the United States has resulted in dramatic reduction in the number of cases of invasive Hib infections.[266] Figure 3 shows the reduction in cases of Hib meningitis relative to licensure dates and ages of use of Hib vaccines (1) and Figure 4 shows sustained low rates of *H. influenzae* invasive diseases (most of which are caused by type b strains) since the introduction of the vaccines. In spite of the availability of vaccine, however, a small number of children remain undervaccinated; risk factors for failure to receive the complete primary series include having a single-parent mother (odds ratio [OR] = 4.3) and household crowding (OR = 3.5).[267] Among the undervaccinated, living with a smoker and other indicators of lower socioeconomic status such as low family income and low maternal education were independent risk factors for Hib disease.

Mortality rates from Hib meningitis declined rapidly after the introduction of PRP-based vaccines.[268] Prior to introduction of the vaccines mortality rates were approximately 1.5 deaths per 100,000 among children 1–4 years old, and in the year following use of the conjugate vaccines fell to 0.11/100,000 in children ≥2 months old.

Some of the decreased incidence rates and mortality rates of Hib infection are related to the role of conjugate vaccines in reducing colonization of *H. influenzae* type b,[149] likely because of mucosal antibody stimulated by immunization with these vaccines.[269] Similarly, reduced colonization reduces the pool of infectious children in the community, and thus, in communities with high vaccine uptake, the vaccines appear to confer a degree of herd immunity by providing protection for unvaccinated children.

The direct and societal benefit–cost ratios for the Hib vaccination program in the United States have been shown to be 3.4 and 5.4, respectively, resulting in $0.95 billion in benefit from the direct cost perspective and 2.09 billion in benefit from the societal perspective.[270]

9.1.1.5. Vaccine Failures. While undervaccination is the likely explanation for continued Hib infection in the United States, a few fully vaccinated children become infected. Evidence from the United Kingdom showed that vaccine immunity waned following the administration of three doses prior to 4 months of age with no booster at age 2 years.[271] Among British children who developed Hib infection in spite of vaccination, a significantly greater proportion of the infecting strains had increased copy number of the *cap* region, suggesting higher virulence of these strains.[272] In addition, children with antibody deficiency syndromes such as X-linked agammaglobulinemia (Bruton's syndrome), IgG subclass deficiency, or partial antibody deficiency with impaired polysaccharide responsiveness are at increased risk of failure to mount a protective immune response to Hib vaccines.

Following successful introduction of Hib vaccines in Alaskan Natives, re-emergence of invasive disease appeared in 1996[112] and appeared to be related to the use of conjugated vaccines that did not stimulate protective levels of antibody until after the second or third dose. The exclusive use of a product (PRP-OMP) that gave high antibody levels after the first dose subsequently reduced Hib disease rates to low levels in this population. Yet the disease rates among rural Alaskan Native children remain higher than rates in urban Alaskan Natives or other US children, suggesting that equity in disease rates may not be achieved in vaccinated indigenous populations without addressing other environmental and household risk factors.[273]

9.1.2. Antibiotic Prophylaxis Against Hib Infection. Non-immune children under age 4 years who are in close contact with an individual with Hib infection are at increased risk of invasive Hib disease. Because close household and day care contacts of an index case have higher rates of Hib colonization,[274,275] the risk of Hib infection is increased in non-immune children attending a day care or living in a household in which a case of Hib infection has occurred.[276,277] Clinical studies have demonstrated the efficacy of rifampin chemoprophylaxis to reduce Hib nasopharyngeal colonization of close contacts of an index case with Hib disease and, thus, decrease the risk of infection in young, non-immune contact children.[278,279]

The prevention strategy using antibiotic prophylaxis is based on decreasing nasopharyngeal colonization of individuals in close contact with a non-immune child who is at increased risk of Hib infection. Because secondary cases usually occur within a week of the presentation of the index case,[280] prophylaxis should be administered as soon as possible. An index child may be persistently colonized with Hib following treatment with an antibiotic that does not reduce colonization.[281] Table 6 outlines the recommendations for rifampin prophylaxis of close contacts to prevent Hib infection in an at-risk, non-immune child.[263]

Prophylaxis is not indicated in households or day care centers with no at-risk children, such as those with no children <4 years old or with no unimmunized children.

9.2. Vaccines Against NTHi

Because NTHi lack capsular polysaccharide on their surfaces, the Hib vaccines do not protect against infections caused by NTHi. Considerable effort has been directed at identifying bacterial surface antigens for use in a NTHi vaccine[282]; candidate antigens have included the adhesins P5 and the high molecular weight protein, OMP 26, P6, and detoxified lipooligosaccharide. A major barrier to use of many NTHi outer membrane components as vaccine agents is their high level of strain-to-strain diversity. A combination vaccine composed of 11 different *S. pneumoniae* polysaccharides conjugated to protein D, a cell surface lipoprotein of NTHi, as a carrier protein showed only limited efficacy in preventing NTHi acute otitis media, 35.5%, €95% CI 1.8–57.4).[283] Because NTHi primarily cause infections of the respiratory tract, novel approaches to vaccine development, such as direct mucosal application or modulation of specific immune responses, may be required for a successful NTHi vaccine.[284]

Table 6. Recommendations for Rifampin Prophylaxis to Prevent Hib Infection

Household contacts of an index case:

* All contacts (defined as persons residing with the index patient or non-residents who spent four or more hours with the index case for at least 5 of the 7 days preceding the day of hospital admission of the index case) irrespective of age in a household in which an unimmunized or incompletely immunized child <4 years old lives
* All contacts in a household in which an immunocompromised child, regardless of the child's immunization status, lives

Day care center contacts of an index case:

* All contacts in a nursery school or child day center in which ≥ two cases of Hib infection have occurred within 60 days

Index case:

* Index cases treated with an antibiotic other than a third-generation cephalosporin and ≥2 years old or in a household with a non-immune child <4 years

10. Unresolved Problems

The introduction of the conjugated *H. influenzae* type b vaccines has resulted in a stunning reduction in morbidity and mortality from infection caused by *H. influenzae* type b—a remarkable public health achievement accomplished through the collaboration of basic scientists, clinicians, vaccine manufacturers, and public health providers. Challenges remain in achieving high vaccine coverage rates in developing countries and sustaining high coverage rates in industrialized countries. In addition, non-typeable *H. influenzae* continue to cause significant morbidity in the form of acute respiratory infections such as bronchitis, otitis media, and sinusitis. Development of a NTHi vaccine has been elusive and continued efforts to identify appropriate vaccine components to prevent non-typeable *H. influenzae* infections are warranted.

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CHAPTER 18

Helicobacter pylori

Sharon Perry, Catherine de Martel, and Julie Parsonnet

1. Introduction

Helicobacter pylori, a corkscrew-shaped, gram-negative rod, resides in the mucous layer of the human stomach. Unrecognized until the 1980s, the organism is extraordinarily common, infecting at least half of the world's population. Once acquired, infection persists chronically, typically continuing in the stomach throughout the host's life. *H. pylori* infection invariably causes acute and chronic gastric inflammation, but in only a minority of cases does it result in overt clinical disease. Yet, the diseases attributed to *H. pylori* infection, peptic ulcer disease and gastric malignancy, are among the world's most important causes of morbidity and mortality in adults. For this reason, the past 30 years have borne witness to furious activity concerning diagnosis, treatment, and prevention of this recently rediscovered pathogen.

2. Historical Background

In 1893 Bizzozero[23] first reported the presence of spiral-shaped organisms in the mammalian stomach. By the 1930s pathologists had linked these spiral bacteria to gastric inflammation and upper gastrointestinal disorders.[57,85,163,253] Later, the organisms were deemed postmortem contaminants, and work on gastric spiral bacteria largely ceased by the 1950s.[239] Some attention continued to be directed to the surprising production of urease within the gastric mucosa, but a specific connection between this enzyme and the previously identified spiral bacterium was not made.[78,178]

In the 1970s, interest in gastric spiral organisms was revived by a few, scattered investigators.[87,302,303] In 1979, Robin Warren, a pathologist in Perth, Australia, began to intensively study the organism and connected it with a common form of gastric inflammation: chronic superficial gastritis.[205] On the strength of this pathological data, Barry Marshall, a gastroenterologist in Perth, treated an infected, dyspeptic patient with tetracycline; the patient's symptoms and histological gastritis completely resolved. From then, Warren and Marshall's research accelerated. By 1982, their group made two major advances: they cultured the organism for the first time (it was named *Campylobacter pyloridis*, but was later renamed *Helicobacter pylori* when taxonomic studies determined it was not a *Campylobacter*) and linked it with peptic ulcer disease.[205] In 1984, after a period of contentious academic debate, Marshall addressed the skeptics by ingesting the organism, thus inducing a self-limited gastritis.[203] Soon thereafter, another investigator repeated the experiment and developed sustained gastric inflammation that did not respond to simple antibiotic regimens.[221] These final experiments convinced most doubters of a role for *H. pylori* in producing gastric inflammation. In 2005, Drs. Marshall and Warren shared the Nobel Prize in Medicine for their pioneering and tenacious work.[202,244]

Since 1984, tens of thousands of articles by thousands of investigators worldwide have been published concerning *H. pylori*. These articles amply document the high prevalence of infection in populations, a causal link to dyspepsia, peptic ulcer disease, gastric cancer, gastric lymphoma, and a protective association with gastroesophageal reflux disease (GERD) and esophageal adenocarcinoma. Numerous studies have shown that many gastric and duodenal ulcers can be cured effectively with antibiotic therapy. As a consequence, in 1994 the National Institutes of Health declared *H. pylori* to be a major cause of peptic ulcer disease and recommended antibiotic therapy as a mainstay of treatment.[42] Others have

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demonstrated that *H. pylori* increases risk of gastric cancer at least 4-fold. In 1994, the International Agency for Research on Cancer declared *H. pylori* a group 1 carcinogen (a definite cause of cancer in humans[135]). Most recently, the declining incidence of *H. pylori* infection has been accompanied by a rapid increase in the incidence of esophageal cancers, leading to new speculations regarding protective effects of infection on upper gastrointestinal disease.[252]

3. Methodology

3.1. Sources of Mortality Data

H. pylori does not invade beyond the gastric mucosa and does not directly cause fatalities. There are, therefore, no mortality statistics available for *H. pylori* infection. Diseases associated with *H. pylori* infection, however, do lead to death. Mortality statistics for peptic ulcer disease have not been systematically collected, although several investigators in the United States and Europe have analyzed data from local areas. These data reveal decreasing mortality rates from peptic ulcer disease over time.[166,299–301] The highest mortality has been observed in persons born at the time of the Industrial Revolution, with a gradual decline in risk since. Regional cancer registries throughout the United States[37,357] as well as in other countries[75,157,241,326,355] provide mortality statistics for gastric malignancies.

3.2. Sources of Morbidity Data

H. pylori infection is not a reportable disease. Information on *H. pylori*-related diseases can be obtained from investigator-generated studies and cancer registries.[17,37,141,166,300,322,357] Because dyspepsia is often treated empirically without diagnosis of the underlying pathology, morbidity data for gastric and duodenal ulcers are considered unreliable.

3.3. Surveys

Surveys are the most widely used method for studying the population distribution of *H. pylori* infection. Household, institutional, and population surveys have been conducted in numerous populations worldwide (see Section 5.1). Serological surveys have been the most widely employed cross-sectional design since, in untreated populations, a positive serological test accurately reflects active rather than past infection. Stool antigen and breath tests are also becoming popular as surveillance tools, particularly in studies of children.[14,99,109,278,292,317]

3.4. Laboratory Diagnosis

Both endoscopic and nonendoscopic methods exist to diagnose *H. pylori* infection; none is perfect. The sensitivity and specificity of the diagnostic methods and their respective pros and cons are listed in Table 1.

3.4.1. Isolation and Identification. *H. pylori* is a relatively fastidious organism that does not thrive outside of its natural environment. Despite this, the organism can be cultured from gastric mucosa if proper measures are taken. Biopsies obtained from the stomach should be rapidly placed in transport medium (such as Stuart's medium with chocolate or broth with supplemental serum) to prevent desiccation. Ground gastric biopsy specimens should then be plated on a solid medium as soon as possible.[123] Delay in culture mandates freezing of the biopsy specimen at 4°C until the time of plating. *H. pylori* requires an enriched environment

Table 1. Diagnostic Tests for *Helicobacter pylori* Infection

Test	Sensitivity (%)	Specificity (%)	Comments	Cost	Reference
Endoscopic					
Histology	90–95	95–98	Based on at least two antral biopsies	$$$	[226, 332]
Culture	70–92	100	Technically demanding; best reserved for research	$$$	[35, 225]
Rapid urease test	88–97	93–98	Endoscopic method of choice for diagnosis	$$$	[167, 212]
Noninvasive					
^{13}C or ^{14}C breath test	>95	>95	^{13}C for children and pregnant women; good for patient follow-up	$	[38, 95, 107]
Stool antigen test (polyclonal – monoclonal)	91–96	93–97	Good for children and for patient follow-up	¢¢	[94, 96]
Serology	80–99	87–96	Good for surveillance; not good for short-term follow-up or children <2 years	¢	[67, 69, 73]

for growth. Many agar bases will sustain the organism if supplemented with blood or serum; antibiotic supplementation in the medium may minimally increase culture sensitivity. Plates should be incubated at 37°C under microaerophilic conditions (10% CO_2 with 98% humidity).[123] *H. pylori* grows slowly and plates may require 7 days incubation to develop recognizable colonies. Presumptive identification can be made if organisms have typical gram-negative, curved morphology on Gram's stain and produce both catalase and urease. In research settings, a more definitive diagnosis can be made with molecular probes and/or electron microscopy.

On rare occasions, *H. pylori* has been cultured from stool and saliva[162,248,321] which is not routinely effective. Another experimental method of obtaining isolates is a gastric "string test," for which sensitivity has been quite variable.[176,284,337]

Culture is the least sensitive but most specific method for diagnosis of *H. pylori*. It is particularly insensitive in laboratories with little experience.[225,332] Because other diagnostic tests are widely available, culture is best reserved for research laboratories with an interest in obtaining isolates for research and/or antimicrobial sensitivity testing.

3.4.2. Other Endoscopic Methods of Diagnosis. Histology is still considered by many to be the "gold standard" for diagnosis. Not only does it reliably identify infection, but it also can provide information on underlying gastric pathology. Because *H. pylori* is not evenly distributed throughout the gastric mucosa, however, at least two biopsies need to be performed to sensitively diagnose infection.[220,226] Routine hematoxylin and eosin staining will usually reveal the organism; the more sensitive but more expensive silver stains should be reserved for inconclusive examinations.[35]

Another commonly used endoscopic test is the rapid urease test. This method exploits the organism's rapid hydrolysis of urea to ammonia and carbon dioxide. A biopsy of the stomach is obtained and placed in a urea-containing medium with a pH indicator. Production of ammonia turns the indicator red and confirms the presence of the organism. The rapid urease test can often be interpreted in less than 1 hour and is substantially less expensive than histology. Nonetheless, it does require endoscopy. Reported sensitivity and specificity of rapid urease tests vary considerably from study to study.[175,206,208] Most, however, show approximately 90% sensitivity with even greater specificity.[167,212]

3.4.3. Nonendoscopic Diagnostic Tests. Three noninvasive methods of diagnosing *H. pylori* are widely used: serology, breath test, and stool enzymatic immunoassay (EIA). The usual serologic assays are quantitative enzyme-linked immunosorbent assays (ELISAs) that measure serum IgG titers against *H. pylori*-specific antigen. In untreated patients—including patients treated with single antibiotics for other purposes—IgG response to *H. pylori* infection is stable over the years, reflecting the chronic nature of infection even in the face of routine antibiotic use.[245] Conversely, 6 months following successful eradication therapy against *H. pylori* infection, antibody titers typically decrease at least 50%.[48,161] This drop in titer appears to be quite sensitive and specific for successful treatment. Unfortunately, a single serum obtained following therapy will not necessarily become quantitatively negative, limiting the use of serologic tests in previously *H. pylori*- treated subjects.

Numerous commercial serologic tests—including in-office rapid tests— are available; the antigens used vary from kit to kit as may the simplicity of the assay methods. Consequently, sensitivity and specificity also vary[73,182]; some kits perform inadequately.[9,332] At their best, in adults and older children, ELISA kits have sensitivity and specificity for active infection exceeding 90% and sometimes exceeding 95%. Sensitivity is considered unreliable in very young children.[50,55,122,148] ELISAs may also have varying performance from population to population, perhaps due to differences among the *H. pylori* strains used for the ELISA and those found in the population.[9,31,52] For these reasons, serological tests should be evaluated in the intended population before being used for epidemiological or clinical purposes.

Serologic tests to measure IgG antibodies against the CagA protein of the pathogenicity island (see Section 7) have also been developed.[22,46] These tests, which target the 128 kDa protein of the *cagA* pathogenicity island (Section 4), have become important in epidemiologic surveys of populations at risk of more virulent outcomes such as gastric cancer, but are less useful in the clinical setting.

Carbon-labeled urea breath tests have become increasingly popular for clinical diagnosis as well as in epidemiologic surveillance. Now approved by the US FDA for use in routine diagnosis, these tests exploit the organism's production of urease to detect active infection following oral administration of ^{13}C- or ^{14}C-labeled urea.[95,156] If *H. pylori* is present in the stomach, the ingested urea is metabolized into ammonia and labeled CO_2. Using either mass spectrometry (^{13}C) or scintillography (^{14}C), the amount of excess CO_2 relative to other carbon isotopes may be measured in the exhaled air of an infected patient within half an hour. Although there is no evidence that the amount of radiation exposure associated with use of the ^{14}C breath test (about 1 uCi residual) is unsafe, the test is contraindicated in pregnant women and children.[38] The ^{13}C breath test uses a stable carbon isotope and is appropriate for use in these groups. Though more expensive than serology,[119,334] the

tests' high sensitivity and specificity make them potentially valuable in surveillance work, as well as in many clinical situations, including pre-endoscopic screening, antibiotic eradication management, and following surgical resection.[39,95] Because false-negative results are possible if the patient has been taking antibiotics, proton pump inhibitors, antacids, or antihistamines that decrease gastric acid secretion,[110] clinicians must be alert to circumstances that make the tests most informative. Several factors, including type of test meal, dosage, type of urea, and clinical (pre- or post-treatment) indication may influence choice of cut-off values for qualitative interpretation.[95] For use in small children, correction for body mass-dependent CO_2 production may improve validity.[155]

Enzymatic immunoassays to detect the presence of *H. pylori* antigen in stool have now become commercially available. A rapid anti-*H. pylori* antibody capture, the test can be performed on fecal samples at room temperature with a colorimetric result obtained in 1–2 hours for the laboratory kits and in a few minutes for some of the new in-office products. In untreated patients, one systematic review estimated weighted sensitivity and specificity, which are often evaluated against the ^{13}C urea breath test, at 91% and 93%, respectively.[96] In a EUROGAST study of 501 untreated patients, sensitivity and specificity were 94% and 92%, respectively, by stool antigen test versus 95% and 98% by breath test when both were evaluated against a combination of biopsy-based culture and urease test.[333] Both polyclonal and monoclonal antibody conjugates are available, with the latter considered somewhat more accurate.[94,128,290] Although considered promising for use in small children, relatively few studies have focused on this population. In 127 children aged 4 months to 17 years, overall sensitivity and specificity of the polyclonal antibody were 95% and 97%, but 80% and 96%, respectively, in children 2–6 years of age; no infants under 2 years of age had positive tests.[269] In a small pediatric series, stool antigen was correlated with non-specific *Helicobacter* sp. DNA amplification of stools. Among 15 sequences from stools 3 were non-*pylori Helicobacter* species,[118] indicating both colonization of children with other species and lack of test specificity.

The stool antigen tests have been evaluated for use in post-treatment evaluations. According to one review of studies in which at least two reference standards were used, positive and negative predictive values were 77% and 89%, respectively, when performed 4–8 weeks following treatment.[97] In Japan, diagnostic accuracy of the stool antigen test was 97%, versus 94% by ^{13}C breath test, in 105 patients evaluated at least 8 weeks post-eradication.[312] As with the breath test, recent use of proton pump inhibitors may be a cause of false-negative results.[128] Stool sample may be stored at 2–8°C for 3 days and at −20°C indefinitely. The relative simplicity and cost-effectiveness of the test make it a useful addition to epidemiologic and clinical investigations, particularly in evaluating populations without frank indications for endoscopy.

Polymerase chain reaction (PCR) can sensitively detect *H. pylori* DNA in the saliva, dental plaque, or gastric juice of persons with gastric infection but is considered primarily a research tool.[34,199,228]

4. Biological Characteristics of the Organism

The genus Helicobacter, recognized with the rediscovery of *H. pylori*, comprises a diverse group of curved, spiral bacteria that colonize the gastric and intestinal tracts of animals as well as humans.[346] Adapted to the human stomach, *H. pylori* is a neutralophilic spiral-shaped, gram-negative rod 2.4–4.0 μm long and 0.5–1.0 μm wide possessing 2-6 flagellae at one end that provide it with corkscrew motility. Under stress, *H. pylori* loses its spiral morphology and first becomes more simply curved and eventually coccoid. Some believe the coccoid forms to be metabolically active, transmissible, but noncultivable, forms of the organism; most recent data, however, suggest that coccoid forms are not viable.[30,64,283]

Approximately 30 different outer membrane proteins (HOPs) have been identified, of which several, most notably BabA, have been implicated as adhesins.[5,25,187,267,291] Expression of adherence factors may be adaptive, with the organism able to exploit both phase variation and host antigenic variation to regulate attachment.[336] In any event, adherence may be necessary to prevent elimination during gastric emptying.

As a neutralophile, *H. pylori* grows optimally at a pH between 6.0 and 8.0. Although it can survive in artificial media at pH of 1.5 if given supplemental urea,[204,305] under more relevant conditions, it is unable to survive at pH $<$ 3.0, or to grow at pH $<$ 6.0 or above 8.3.[281] While many neutralophilic gram negatives can survive acid shocks (pH $<$ 3.5) during transit of the stomach, *H. pylori* is unique in its ability to colonize this environment. It appears to do this, at least in part, through prodigious production of urease, which it produces far in excess of other neutralophiles.[219] Accounting for 10–15% of proteins by weight, this enzyme, a 300- to 625-kDa nickel-containing hexamer, hydrolyzes urea to CO_2 and ammonia.[329] Crystallography has revealed a large 1.1 MDa supramolecular assembly consisting of 12 catalytic units active to a pH of 3.[117] Urease is primarily a cytoplasmic protein,[286] although it may also be found on

the surface of the bacteria due to lysis or other mechanisms that are poorly understood.[201,281,304]

The physiologic role of *H. pylori* urease appears to be both novel and complex. In animal models, urease is essential for *H. pylori* colonization of the stomach,[10,63,328] and in gene expression studies survival of acid shocks <3.0 depend on urease production.[336] The *H. pylori* urease gene cluster consists of seven essential genes, organized into two primary segments, UreA and UreB, and five accessory genes,[3] with regions of UreB appearing to differ significantly from other known urease.[117] Commonly thought to be constitutively expressed,[281] recent evidence suggests that urease activity may be more controlled.[304] The UreI protein, a proton-gated urea channel that may be unique to *H. pylori*, now appears to play a critical role in generating a rapid urease response to shifting micro-environmental conditions.[262,344] However, since urease production accounts for a considerable portion of the organism's energy expenditure, *H. pylori* urease probably serves other essential functions. Damage to the gastric mucosa induced by urease and/or ammonia may provide the organism with necessary nutrients or an environment appropriate for attachment and growth. Alternatively, urease-induced ammonia may provide protection from the innate immune system and may also be important for nitrogen assimilation by the organism.[90,347] Urease may also disarm the innate immune system during early stages of infection.[101] Nonurease genes may also be involved in acid regulation.[345] This suggests a tightly coordinated and adaptive acid resistance system.[282]

One of the most important developments of the past two decades has been the identification of the Cag pathogenicity island (PAI), named after one of its proteins—the 128 kDa CagA (cytotoxin-associated gene A) protein—that elicits a strong IgG response and thereby serves as a marker for the presence of the PAI.[24] Found in approximately 60–70% of US isolates the PAI is a large chromosomal cassette flanked by 31 base pair DNA repeats that encode a type IV secretion system (TFSS) that in addition to promoting adhesion[124] and translocation[238,250] is involved in disruption of signaling pathways of epithelial cells.[8,25,251,287]

Another important protein of *H. pylori* is its vacuolating cytotoxin which is encoded by the VacA gene. This 88-kDa secreted protein has structural homology with the IgA proteases of *Neisseria* and *Haemophilus*.[285] It received its name from the vacuole formation it induces in tissue culture cells.[45] In mouse models, oral administration of purified cytotoxin not only induces vacuolation of gastric mucosal cell but also results in inflammation and gastric ulceration.[318] Recent studies have suggested that the p55 domain plays an important role in epithelial binding.[43] All strains of *H. pylori* possess the cytotoxin (*VacA*) gene, although expression of a functional, virulent protein occurs in only 50% of strains.[44] Strains possessing VacA alleles of the type s1/m1 form appear to have the greatest vacuolating activity[43]; such alleles are typically found in isolates with a PAI.

H. pylori strains exhibit enormous genetic diversity.[4,26,133,314] *H. pylori* has been completely sequenced twice[6,233,324] and has a 4.5 kb genome with approximately 1,400 informative sites. Overall, only 94% of genetic content was shared by J99 and 26695, and 7% constituted by genes unique to one or the other.[6] One group sequenced 370 gene fragments in 27 geographically separate populations, finding that of 1,531 genes present on average, only 1,111 existed in all strains.[115] By fingerprinting methods, it is unusual to find identical strains in different subjects. In one report, clinical isolates were obtained from the same patient 6 years apart. Compared with the original J99 strain, sequencing of the new isolates revealed 3% variation compared with 22% when isolates of different individuals were compared.[139] In another report using whole-genome DNA microarray to compare 21 pairs of isolates taken from the same patient over time, the majority of genetic changes were classified as homologous events, rather than net loss or acquisition of genes.

The tendency for frequent recombination involving relatively short fragments makes the organism useful for ancestral genetic studies. Strain variants deduced from phylogenetic models appear to parallel the major routes of human migrations in modern times.[70,147] The PAI appears to bear the signature of a relatively recent horizontal acquisition.[26] Thus, *H. pylori* may have been colonizing humans since the origins of modern civilizations, utilizing genetic variation to adapt to individual hosts as well as populations.

Though *H. pylori* is by far the most important helicobacter in humans, some non-*H. pylori* helicobacters may also colonize humans.[83]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

H. pylori has been present in humans for tens of thousands of years[70] and still infects half the world's population. Acquired primarily in childhood, the infection persists throughout life. Many seroprevalence studies of *H. pylori* have been conducted worldwide and the organism has been found wherever it has been sought. Prevalence of infection, however, varies widely between and within population groups. Prevalence in developed countries

has been declining in recent decades and is considerably lower than in developing countries. Prevalence of infection within populations also varies with age and socioeconomic status.

Figure 1 compares age-specific prevalence rates from several selected population-based studies. In developing countries (Section A), a majority of the population is infected by age 10 years. In developed countries (Section B), prevalence of *H. pylori* is lower. Infection in children in these countries is now rare, except in immigrants from poorer regions of the world.[16,62,255,258,327] Of course, there are some exceptions to these two main patterns. For instance, a seroprevalence survey conducted in a small isolated Nepalese village showed a low rate of infection, despite the low socioeconomic level and the poor level of hygiene and sanitation.[145] Within countries, the proportion of infected patients with antibodies to CagA appears to be relatively constant with respect to age and gender.[254]

Because acute *H. pylori* infection virtually always passes undiagnosed, incidence cannot be estimated from clinical data. However, some information on incidence can be gleaned from longitudinal seroprevalence surveys. In some surveys, an exponential increase in seroprevalence with age suggests a steady incidence of infection over time.[242] For example, in whites from the United States, prevalence data correspond closely to a 1% per year rate of infection. In contrast, prevalence in one African-American population conformed to an incidence of 3% per year.[109,242] Most prevalence data, however, do not support a constant incidence rate.

Because of the difficulties in determining incidence based on clinical illness or prevalence data, investigators have repetitively tested for *H. pylori* infection in population cohorts followed over time (Table 2). Despite differences in study design, the data from developed countries are relatively consistent in their estimations of seroincidence. In adults (Table 2A), a mean of 0.4% of subjects converted from *H. pylori* seronegative to seropositive per year.[242] Coincidentally, the seroincidence rate observed in prospective studies of adults closely matches the incidence of reinfection seen in treated patients. This suggests that prior infection with *H. pylori* neither protects against nor predisposes to reacquisition of the organism. In studies evaluating children (Table 2B), incidence rates are higher than in adults, with the highest rates of acquisition observed around age 2 years and most infections acquired by age 5 years.[113,188,277,280] In very young children from high-risk groups, frequent transient infections (a positive test followed by a negative test) before the age of 2 years have also been reported.[104,153,256,277,320] What proportion of these phenomena represents true transient infection versus single false positives in subjects with low pretest probability remains controversial.[234,260,276]

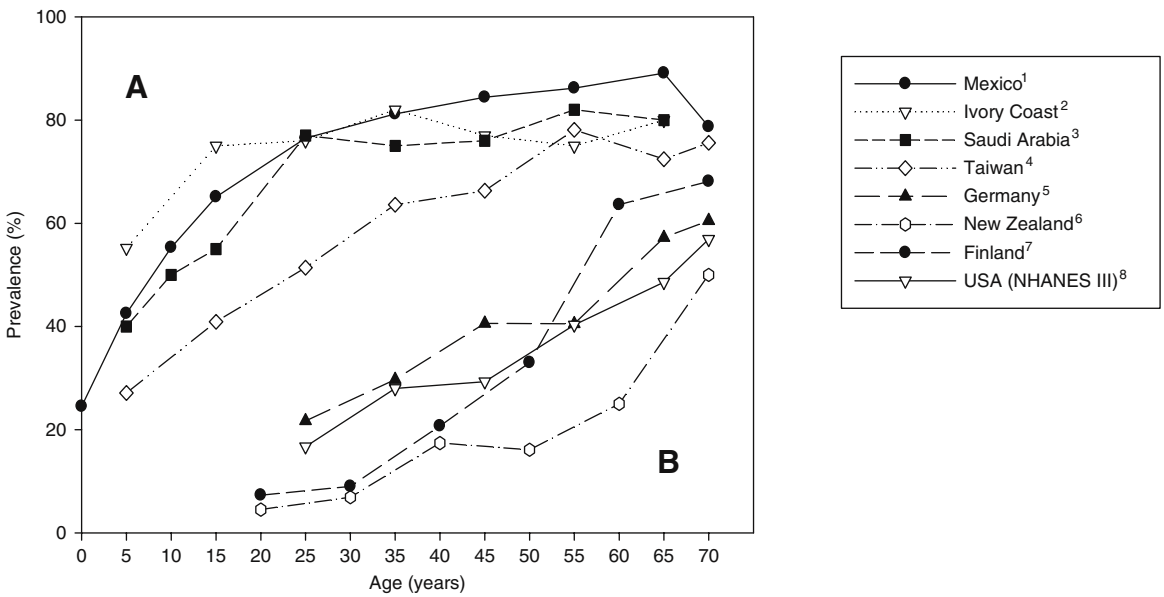


Figure 1. Prevalence of *H. pylori* in healthy persons by age in (A) developing countries and (B) developed countries. References: **A**: Mexico[325], Ivory Coast[210], Saudi Arabia[7], Taiwan[317]; **B**: Germany[288], New Zealand[41], Finland[160], United States[68].

Table 2. Prospective Studies of *H. pylori* Incidence[a]

Population	Test	Follow-up (mean years)	New infections/total studied	Incidence (%/year)	Reference
A. Adult studies					
Dutch endoscopy patients	Serology	11.5	2/59	0.3	[164]
Australian adults	Serology	21	6/89	0.3	[47]
American epidemiologists	Serology	8.5	11/278	0.5	[245]
Canadian cohort	Serology	1.5	3/196	1.0	[338]
Finnish endoscopy patients	Serology	15	12/181	0.4	[297]
Japanese cohort	Serology	6.7	5/86	1.0	[165]
Finnish adults (Vammala)	Serology	21	9/126	0.3	[160]
B. Pediatric studies, age at baseline					
Finnish infants, 0–2 years	Serology	2	10/174	2.8	[13]
Finnish children, 3 years	Serology	9	2/70	0.3	[12]
Irish children, 2–4 years	UBT	4	20/279	2.1	[280]
Japanese cohort, 6–19 years	Serology	5.4	5/86	1.1	[165]
US Bogalusa Heart Study, 1–3 years	Serology	21	40/206	1.4	[188]
US, Bogalusa Heart Study, 7–9 years	Serology	12	38/171	1.9	[194]
US Northern California immigrant homes, 0–2 years	Stool	1/4	15/287	21	[261]
Swedish infants, 6 months	Serology	10.5	40/294	1.7	[113]
Turkish children in Germany, 1–4 years	Stool	1	7/100	5.3	[278]
Bolivian children, 1–6 years	Serology	1.3	44/188	18	[100]
U.S.-Mexican border newborns, 0 years	UBT	2	128/468	20	[104]
Ethiopian children, 2–4 years	Serology	2.5	44/87	24	[180]

[a]Adapted and expanded from Parsonnet, J. (1995). The incidence of *Helicobacter pylori* infection, *Aliment. Pharmacol. Ther*, *9*(suppl. 2), 45–51.
Note: Serology: IgG antibody test; UBT: ^{13}C urea breath test; Stool: stool antigen test

5.2. Age and Birth Cohort

In Western countries, older age groups consistently exhibit higher prevalence of infection than younger generations. A disproportionately high prevalence of *H. pylori* infection among older persons may reflect one of three phenomena: an age effect (higher acquisition of infection at older ages), an age cumulative effect (steady incidence of infection throughout life), or a birth cohort effect (older individuals may have been born in times when risk of infection was greater). A birth cohort effect explains better how low incidence rates documented in recent prospective trials can be reconciled with high cross-sectional prevalence. Numerous cross-sectional studies that examined populations over time demonstrated a decreasing prevalence of *H. pylori* infection with increasing birth year.[160,165] It is now generally agreed that *H. pylori* incidence has decreased over time in industrialized countries in parallel with improving hygiene and socioeconomic status.

As mentioned above, prevalence of *H. pylori* infection increases with age in all population groups. An exception to this is seen in the advanced elderly, particularly in developing countries. Serosurveys suggest that the very old may have lower prevalence of *H. pylori* than younger persons.[315] It has been speculated that this is due to advanced atrophy lesions that occur with older age. Since *H. pylori* can only survive adjacent to gastric epithelium, destruction or replacement of gastric mucosa by metaplastic intestinal epithelium might result in loss of infection.[92]

5.3. Gender

Men have higher rates of both duodenal ulcer disease and gastric cancer than do women. Yet, while *H. pylori* infection is thought to cause both diseases, studies have shown conflicting results when comparing *H. pylori* prevalence between genders. A recent meta-analysis of previously published large population-based studies showed a small but significant and homogeneous elevated risk of infection among adult men concordant with their elevated risk for *H. pylori*-related diseases. This male predominance was not apparent in studies of children.[54] This finding portends either a greater loss or greater acquisition of infection in female adolescents or young adults.

5.4. Race–Ethnicity

Serosurveys of *H. pylori* infection have identified pronounced differences in prevalence among racial/ethnic

population groups. In the United States, higher prevalence of *H. pylori* has been reported in African-American and Hispanic populations compared to non-Hispanic white populations.[109,129] In the largest and most representative study—a cross-section of the US adult population with overall prevalence of 33% (NHANES III, 1988–1991)—age-adjusted seroprevalence was 62% in Mexican-Americans, 53% in African-Americans, and 26% in non-Hispanic whites.[68] Outside of the United States, racial–ethnic variations in *H. pylori* prevalence also have been observed. In Taiwan, the highest seroprevalence (63.4%) was found in a rural indigenous population and the lowest (40.5%) in an urban area inhabited primarily by Hakkas.[317] The indigenous Maori of New Zealand also have higher (39–70%) infection rates than white New Zealanders (15%).[222] This discrepancy, though consistent, has varied in magnitude with the age and other characteristics of study populations.[84,254] Although an early study found the opposite,[59] Western Australian aborigines may have infection rates two to three times higher than non-indigenous Australians.[348] In Belgium, non-Caucasian children were more likely than Caucasian children to be infected.[28] Similarly, in Malaysia, a country of diverse Asian races, Malays were reported to have significantly lower infection rates than Chinese or indigenous Indian groups.[102] Although comparative studies are lacking, indigenous populations of the Americas, where studied, may have much higher rates of infection than non-indigenous groups.[21,256,274]

The reasons for racial–ethnic differences are not entirely understood. Poor socioeconomic conditions during childhood, as measured by household crowding and parental income, are thought to play an important role (see Section 5.5). US immigrant groups, for example, tend to have infection rates reflecting country of origin,[255,327,340] although it is not clear whether these differences are sustained over multiple generations after accounting for parents' infection, education, and household crowding.[236,327] Socioeconomic conditions cannot, however, completely explain observed differences and a genetic component cannot be entirely excluded.[273] A comparison of *H. pylori* concordance in monozygotic and dizygotic Swedish twins reared apart suggested that a proportion of infection may be attributable to genetic factors.[189]

5.5. Socioeconomic Status

Virtually all studies to date have shown an inverse relationship between *H. pylori* infection and socioeconomic status. A number of markers have been used as indirect measures of socioeconomic status including level of education, crowding, sharing of beds, and availability of indoor plumbing or hot running water in the home. In one large study (the EUROGAST serosurvey of 3,194 asymptomatic individuals in 17 countries), *H. pylori* seroprevalence showed a significant inverse association with educational level in 11 of the 17 population groups[66]; in the remaining 6 population groups, the inverse relationship remained but did not attain statistical significance. In Wales, age-adjusted prevalence of *H. pylori* was also found to be highest in the lowest social classes (62%), less in the middle class (57%), and lowest in the higher class (49%).[129]

Socioeconomic status during childhood may serve best as an indicator of *H. pylori* risk. For example, in 150 healthy African-Americans and Hispanics between the ages of 19 and 49 years, a combined index of parental education, occupation, and income was a strong predictor of *H. pylori* infection. About 85% of adults raised in the lowest socioeconomic stratum had infection compared to 11% in the highest stratum. The association remained after adjustment for age and current social class.[191] Studies of US high-income versus low-income families in Arkansas and in Texas support this finding.[76,109] In Japan, a country that has undergone dramatic economic development since the Second World War, 5,854 adults were asked to describe household living conditions before age 10 years. Lack of modern plumbing facilities and household crowding were significantly associated with contemporary *H. pylori* seroprevalence rates.[330] Studies from Korea and Mexico similarly link household hygiene in childhood with adult infection.[193,325] By contrast, infection rates among urban homeless adults in Toronto, Canada,[134] were similar to other Canadian adults[254] and not associated with common stomach complaints. These data reinforce incidence and prevalence data (Section 5.1) that point to unsanitary living conditions in childhood as a critical factor for acquiring infection.

6. Mechanisms and Routes of Transmission

Because acute infection invariably passes undetected (Section 8), acute *H. pylori* infection has rarely been diagnosed outside the experimental or iatrogenic setting. In the absence of such acute infections, particularly outbreak-related infections, the means of *H. pylori* transmission have had to be deduced using indirect epidemiologic methods. The majority of the resulting data support person-to-person transmission as the dominant, if not only, mode of transmission.

6.1. Person-to-Person Transmission

Epidemiologic studies of *H. pylori* transmission vary greatly in their design and implications. Many cross-sectional studies, comparing infection rates between groups with different risk factors, have been conducted. Seropositivity rates are higher than expected in close communities where hygiene is suboptimal, such as institutions for the disabled[20,32,149,169,272] and orphanages.[196,257] Infection rates also cluster within families, particularly if a child is the index case.[60,150,192,214,227] In population surveys, familial clustering is a particularly strong feature of community distribution, and this phenomenon has been observed worldwide, despite widely varying socioeconomic conditions.[143] In Belgium, investigators identified a 2.7-fold increased risk of *H. pylori* among first-degree family members of infected, symptomatic pediatric patients compared to the general population (48.6% compared to 17.8%).[29] In a Northern Italian community of 416 families, 44% of children with two infected parents were infected, compared with 30% with one infected parent and 21% with no infected parents.[59] Vietnamese children from 135 extended family homes were over twice as likely to be infected if the mother was infected and four times as likely if both parents were infected.[229] In Brazil, infection among preschoolers was associated with infection status of siblings as well as parents.[275] Other groups report similar results.[2,142,201,203,267,324,334] Patterns of familial risk have also been corroborated in some prospective studies. In a 9-year follow-up study in Japan, seroconversion was observed only among children of *H. pylori*-infected mothers.[195] In Benin, seroconversion of preschoolers was associated with having infected siblings as well as use of open water containers.[99] Outside of the family setting, attending day care[349] or school[323] with infected classmates does not appear to be associated with increased risk of infection. Military personnel deployed overseas under close field conditions may be at increased risk of developing infection in adulthood.[316]

Within households, infection rates have also been related to sibship size, birth order, and current number of children in the family, factors that serve as markers for increased risk of person-to-person transmission within households.[89,103,317] Crowding within households particularly engenders greater risk for infection. Bed-sharing among children, often associated with household transmission of enteric pathogens,[259] is an especially hazardous practice whether documented retrospectively[191,211,342] or concurrently.[72]

In DNA fingerprinting studies, strains of *H. pylori* are more likely to be similar within families than between unrelated individuals.[15,120,152,271] Strains are most closely related among siblings.[339] Among 39 families, 29 of 36 (82%) sibships had identical strains, compared with 10/18 (56%) mother–offspring and 0/17 (0%) father–offspring relationships.[152] In a small cohort series, 5/44 infants who developed infection in the first 5 years of life had strains identical to the mother[159]; however, neither sibling nor fathers' infection status was determined. Sibling strain concordance could represent recombination of a maternal strain, horizontal acquisition, or both.

Three mechanisms of person-to person spread are widely evaluated: fecal–oral, oral–oral, and gastro-oral (defined as iatrogenic transmission through introduction of infected instruments into the stomach) transmission. In favor of a fecal–oral route, one investigative team isolated *H. pylori* from feces of both children and adults, demonstrating that *H. pylori* can pass through the gastrointestinal tract and remain viable.[146,321] In another study using adult volunteers, cultivation was possible only from induced or catharctic, but not normal, stools.[248] In one household incidence study, exposure to gastroenteritis in an *H. pylori*-infected contact was associated with a 4-fold increase in risk of new infection, although exposure to diarrhea alone without vomiting could not be independently linked to new infection.[249] In addition, sewage workers do not appear to have excess risk compared to community controls.[86,140]

Patterns of *H. pylori* infection are perhaps more consistent with oral–oral transmission, particularly in the setting of vomiting or regurgitation. *H. pylori* is cultured reliably from vomitus.[36,177,248] In one comprehensive volunteer series, *H. pylori* (often in large numbers) was cultured from all 80 vomitus samples collected from 16 subjects, and also from a portion of aerosol samples obtained at distances up to 1.3 m, while diarrhea and saliva yields were substantially lower.[248] In the aforementioned household study, exposure to an *H. pylori*-infected household contact with vomiting resulted in a 6-fold increase in risk for new infection.[261] A higher incidence of *H. pylori* infection following outbreaks of gastroenteritis in an institution for the intellectually disabled[174] may consistently be oral–oral as well as fecal–oral transmission. Further support for oral–oral transmission in the setting of vomiting comes from a case–control study in which vomiting siblings and siblings of vomiting index children were 2.4 times more likely to be infected than children not exposed to vomiting.[185] In addition, two case reports—one involving two new infections following vomiting illness of an infected family member,[214] and a second following mouth-to-mouth resuscitation of a person with vomit in the mouth[77]—support this hypothesis.

In the absence of vomiting, it is unclear whether oral–oral transmission occurs. *H. pylori* DNA has frequently been recovered by PCR from both saliva and dental plaque.[162,199]

Only on a rare occasion, however, has *H. pylori* been actually cultured from the mouth.[74,162,248] If oral–oral spread is common, however, one would expect rates of infection among dental workers to be elevated compared to age-matched controls. This was not the case in one cross-sectional study.[190] In a small nested case–control study, Japanese dental professionals had an elevated, but non-significant, risk compared to controls.[207] There is also little concordance of infection among married couples in whom oral–oral transmission might be expected, by serology[258] or by PCR-restriction fragment length polymorphism analysis,[184,308] and treated spouses are not likely to be re-infected by an infected spouse.[49]

The only definitive mode of transmission is gastro-oral. Cases of *H. pylori* have been definitively linked by fingerprinting to transmission via endoscope,[172] and viable organisms can be cultured from gastric juice.[358] Also, outbreaks of *H. pylori*- associated hypochlorhydria have occurred with endoscopy and gastric pH testing in experimental subjects[106,240,270] (see Sections 6.3 and 8.1.1). Of course, this mechanism can explain only a small number of the billions of cases worldwide.

Thus, although the precise mechanism of transmission remains incompletely characterized, it is likely that *H. pylori* employs all three pathways to exploit the milieu of infectious gastroenteritis in close communities. As acute infection is associated with non-specific symptoms of vomiting and diarrhea,[111,138,249,261] it may promote itself during household transmission of common enteric pathogens, with young children serving both as sources and targets of new infection.[248,261] This might explain its persistence in human populations, why acquisition, including repeated acquisition, occurs primarily in early life, and why infection clusters so strongly within families.

6.2. Transmission from a Common Environmental Source

While the preponderance of evidence supports person-to-person spread of infection, some studies in developing countries have reported increased risk of infection in association with exposure to contaminated water,[132,142,154] food, or animals.[36,130] However, other studies are conflicting.[2,100] Numerous studies detecting *H. pylori* DNA in water samples or biofilms by PCR provide only circumstantial evidence of transmissibility.[341] While fecally contaminated water cannot be ruled out as a reservoir in developing countries, the organism has been cultured once under technically demanding conditions from contaminated water,[183] and never from natural water sources.[19] It remains unknown whether the coccoid form commonly observed when exposed to water is viable.[30] *H. pylori* has been isolated from monkeys,[61,82] cats,[121] and houseflies.[116] Conversely, some non-*H. pylori* helicobacters that colonize domestic animals are found in humans.[83] However, no evidence for a zoonotic route of transmission has been reported. Foods of animal origin also seem an unlikely source since vegetarians and meat eaters have similar rates of infection.[129,343]

6.3. Iatrogenic Transmission

Though rare, iatrogenic transmission via endoscopy has been well documented through the isolation of identical strains of *H. pylori* from patients undergoing endoscopy in the same suite. Transmission has been estimated to occur in 0.3% of endoscopies when the endoscope is mechanically cleaned with detergent and ethanol alone.[172] More rigorous cleansing methods, however, prevent transmission.[74,144,235]. Endoscopists and endoscopy nurses appear to be at particularly high risk for infection, leading some to call *H. pylori* an occupational hazard.[40,179] Observance of hospital infection control procedures, however, may mitigate this risk.[232]

7. Pathogenesis and Immunity

Because acquisition of *H. pylori* infection passes unrecognized, little is known about host and/or bacterial factors required for establishing infection. What is known derives largely from experimental or iatrogenic infection. Two self-infection studies are particularly illustrative. To prove the pathogenicity of *H. pylori*, in 1985, Barry Marshall neutralized his gastric pH with histamine blockers (first experiments without histamine blockers failed) and then ingested 10^9 *H. pylori* organisms. Gastroscopy with biopsy on day 10 confirmed infection and revealed antral gastritis. This gastritis resolved spontaneously by day 14. No antibody response developed. Arthur Morris repeated this self-experiment ingesting 3×10^5 *H. pylori*. Biopsies done on day 5 revealed severe inflammation of the antrum with normal fundus.[221] Fasting gastric pH was 1.2 at that time but had increased to 7.6 by day 8. An initial, transient IgM seroconversion was followed by subsequent development of both IgA and IgG antibody against *H. pylori*. Following a 4-week course of bismuth, biopsies revealed no organisms and resolution of inflammatory changes. Biopsy 1 year after ingestion, however, showed persistence of both *H. pylori* and chronic inflammation despite lack of symptoms.[223] The infection and pathological changes remained for over 3 years

until combination antimicrobial therapy successfully eradicated the infection.

These data, in conjunction with a study of experimental infections of volunteers (see Section 8.1), provide almost all that is known on incubation period (estimated 3–7 days).[111,203,221] They also support a hypothesis that there are "windows of opportunity" (such as periods of hypochlorhydria) when exposed persons are at increased risk for establishing infection. Given the high, but not universal, prevalence of *H. pylori* throughout the world, however, it seems likely that many are exposed who do not develop chronic infection.

Indeed, to cause infection, *H. pylori* must survive the highly acidic environment in the stomach and find a hospitable place to replicate. It does these tasks through prodigious production of the enzyme urease,[218] which surrounds the organism with an ammonia cloud, and by using its multiple flagellae to propel it into a protected niche within the mucus overlying the gastric epithelium.[65] Most organisms swim freely in the mucus layer but a minority adhere to the mucosal epithelium through a variety of microbial adhesins and epithelial receptors.[237] This adherence to gastric tissue appears to be highly specific and is related to features of both the host (e.g., blood group type) and the bacterium (e.g., strain).[33,181,335,336] Once established, *H. pylori* lives adjacent to gastric epithelium on the surface or in the mucous layer. Colonies are most commonly observed in the antrum, which lacks acid-secreting parietal cells, although it may also colonize the corpus. Infection is always accompanied by acute and chronic inflammatory changes in the gastric submucosa. The characteristic, histological lesion has been termed superficial active gastritis, type B gastritis, or, most recently, *H. pylori*-associated chronic gastritis with polymorphonuclear cell activity.[264] In the vast majority of infected patients, lymphoid follicles (mucosal-associated lymphoid tissue) also develop.[93] With rare exception, inflammation is accompanied by elevations in serum IgG against several *H. pylori* antigens including the urease subunits and, when expressed by the bacterium, the vacuolating cytotoxin and the CagA protein. *H. pylori* infection also elicits an IgA response in two thirds of infected persons.[161]

Inflammation is thought to be mediated by bacterial and host factors. Although all infected hosts show some degree of chronic and acute inflammation, the inflammatory responses are particularly pronounced in persons infected with strains that contain a cassette of genes named the Cag pathogenicity island (PAI).[263] A functional Cag PAI encodes a type IV secretion system that injects the CagA protein into epithelial cells causing tyrosine phosphorylation of a 145 kDa protein, cytoskeletal changes, breakdown of the epithelial gap junctions, and induction of epithelial cytokines.[224] A functional vacuolating cytotoxin (VacA) further augments the inflammatory response by inducing apoptotic cell death.[91] Inflammation also depends on the host's alleles of specific inflammatory cytokines, e.g., IL-1β, IL-10, and TNF-α.[266]

Although *H. pylori* elicits both a vigorous Th1 cell-mediated immune response in the gastric mucosa and IgG and IgA antibody responses,[307] there is no known natural protective immunity. Reinfection rates are perhaps minimally lower than primary infection rates within the same populations.[243] Vaccines that use adjuvants to boost the natural immune response are being explored. However, even in animals, protective immunity remains poorly understood and neither completely prevents colonization nor mitigates gastric inflammation.[1]

H. pylori-related gastritis can progress to a variety of clinical diseases. The pathogenic mechanisms for the variable outcome of infection, i.e., asymptomatic, ulcer, or cancer, are largely unknown. Simplistically, there appears to be a dichotomy in host response that is distinguished by the location of infection within the stomach and the associated acid response.[294] Some hosts develop predominantly antral gastritis with normal to high acid output. Infection in these individuals may progress to gastric metaplasia in the duodenum and subsequently duodenal ulcer disease. Gastric metaplasia allows *H. pylori* colonization of the duodenal mucosa, where its mucinases and other exoproteins can weaken the mucous barrier in the duodenum, fostering development of ulcers.[27,105,313] Such progression may be enhanced by environmental factors such as smoking or use of nonsteroidal anti-inflammatory agents. In contrast, other hosts develop with multifocal atrophic gastritis involving corpus and antrum with low acid output.[294] Infection in these individuals may progress to intestinal metaplasia, gastric ulcer, and cancer. These dichotomous responses rarely overlap, i.e., those with duodenal ulcer disease appear to be protected from gastric malignancy.[296,298] It is highly plausible that this dichotomy is attributable to differences in host genetics. For example, variations in interleukin 1β and interleukin 1 receptor antagonist genes are associated with both gastric acid secretion and disease outcome.[266] In contrast, the different outcomes are unlikely to be explained by the infecting strain; in both arms of the dichotomy, strains with the PAI and a functional cytotoxin contribute to disease outcome.[33,45]

Research on *H. pylori* as a cause of gastric cancer is proving informative for understanding carcinogenesis more generally. Chronic inflammation secondary to infection has long been known to predispose to carcinomas at the inflamed site. Inflammation typically induces epithelial cell death and accelerates cell proliferation, critical factors in mutation.

Inflammation also induces oxidative bursts of free radicals that potentially can damage DNA. Both enhanced cell proliferation and free radical formation have been observed with *H. pylori*-associated gastritis.[51,186]. Experiments in a mouse model suggest that inflammation also results in recruitment of bone marrow-derived stem cells to the site of injury.[131] Intriguingly, it is now posited that these stem cells, rather than epithelial cells, may be the progenitors of the carcinomatous tissue.

Because of its rarity, less is known about the pathogenesis of *H. pylori*-associated gastric lymphoma than about carcinoma. It appears, however, that *H. pylori* induces T-cells to stimulate B cell proliferation. These B cells are also stimulated by autoantigens in the gastric mucosa that result from cross-reactivity with *H. pylori* antigens. Over time, the lymphoproliferative response can lead to an accumulation of mutations within the B cells. In the early stages of this process, this proliferation can be mitigated by *H. pylori* eradication and the tumor will regress. At later stages, however, accumulated mutations can induce cell growth that is no longer responsive to *H. pylori* eradication.[137]

8. Patterns of Host Response

In the absence of specific antimicrobial therapy designed to eradicate the organism, infection with *H. pylori* typically remains in the stomach for decades, disappearing only in a minority of infected hosts.[88] Genetic fingerprinting analyses demonstrate persistence of the specific strains colonizing the individual.[88,173] Repeated serological testing over time also demonstrates that, in nonelderly adults, the serum IgG titer remains constant in the absence of specific eradication therapy.[171,245] In older adults, however, *H. pylori* colonization and serologic response may diminish or disappear over time in association with increasingly abnormal gastric histopathology.[158] Although the majority of infected individuals are asymptomatic, a spectrum of syndromes and diseases, including dyspepsia, peptic ulcer disease, gastric adenocarcinoma, and lymphoma, have been attributed to *H. pylori* infection. Given the wide variety of outcomes, cofactors such as genetic predisposition, smoking, alcohol, and diet undoubtedly play important roles in determining infection outcome.

8.1. Clinical Spectrum of Disease

Despite histological evidence of inflammation, the majority of individuals infected with *H. pylori* are asymptomatic. For example, in one study, 74% of 57 *H. pylori*-positive healthy blood donors were asymptomatic, whereas 26% reported dyspepsia-like symptoms. These symptoms were similar to those of the *H. pylori*-negative individuals.[127] Despite the lack of a defined, organism-specific clinical syndrome, *H. pylori* is clearly a cause of important clinical diseases. Intriguingly, it has also been touted as potentially protective against other disease processes.

8.1.1. Acute Symptomatic Gastritis. What is known about acute symptoms of *H. pylori* comes largely from experimental infections. In his self-inoculation experiment, Barry Marshall was asymptomatic until day 7 when he developed epigastric discomfort, vomiting, and irritability.[203] Three days after ingesting 3×10^5 *H. pylori*, Arthur Morris developed severe epigastric pain, stomach cramping, nausea, vomiting, and insomnia. Mild abdominal discomfort persisted for approximately 11 days after which all symptoms resolved.[209] An *H. pylori* challenge study conducted in preparation for a vaccine trial has now bolstered the clinical observations of the two pioneering self-inoculations. Among 20 subjects given *H. pylori* after acid suppression, 18 developed infection. All of these developed mild to moderate gastrointestinal symptoms—most commonly dyspepsia and abdominal pain; vomiting occurred in two. Symptoms began in the first week and peaked in the second week.[111]

Acute *H. pylori* infection has also occurred in epidemic form.[98,270] Two endoscopic studies were marred by outbreaks of acute gastritis illness and hypochlorhydria. In the larger of the two studies,[270] an increase in pH was detected in the previously healthy volunteers approximately 1 week after undergoing the gastric secretion studies. This was accompanied by transient epigastric pain, nausea, and vomiting in half of the subjects; the remaining subjects stayed asymptomatic despite increases in gastric pH. Retrospective analysis of biopsy and serum specimens in both series suggested that *H. pylori* was the etiologic agent of these outbreaks.

With naturally acquired infection, much less is known about symptoms. In a study of 1,752 persons followed for new *H. pylori* infection, no distinct symptoms were recognized in 30 patients with newly acquired infection.[261] In a serological study of epidemiologists tested for *H. pylori* infection on two occasions spanning a mean of 8 years, chronically infected subjects had similar symptoms to uninfected subjects. Persons who seroconverted in the 8-year interval, however, had significantly higher rates of self-described upper gastrointestinal symptoms.

8.1.2. Non-ulcer Dyspepsia. The role of *H. pylori* infection in non-ulcer dyspepsia (NUD) remains controversial. Exploring the relationship is problematic because of the wide variety of symptoms that fall under this catch-all term. Differences in the selection of patients for studies and

the method of diagnosis of *H. pylori* infection make comparison between them difficult. Some studies have shown a greater prevalence of *H. pylori* infection in patients with NUD, while others have not. Moreover, while some longitudinal studies have shown symptomatic improvement in dyspeptic patients after eradication of *H. pylori*, the results are not uniform.[168] The seeming inconsistencies may reflect the lack of control groups standardized for other known risk factors for *H. pylori* infection.[310] A Cochrane review of 17 randomized, placebo-controlled trials, however, revealed a 10% risk reduction in non-ulcer dyspepsia with *H. pylori* eradication.[216] Overall, 14 patients required treatment to cure 1 case of non-ulcer dyspepsia. The authors of this review drew no definite conclusion about whether this "modest benefit" warranted screening and treatment but rather encouraged further study.

8.1.3. Peptic Ulcer Disease. *H. pylori* is an important cause of both duodenal and gastric ulcers (1994). Although "no acid, no ulcer" is still an accepted dogma in duodenal ulcer pathogenesis, *H. pylori* is similarly critical. In some studies, over 90% of persons with duodenal ulcer disease had concomitant *H. pylori* infection and the attributable proportion was >90%.[11] In countries where *H. pylori* prevalence is lower and NSAID use is higher, the percent of ulcers with associated *H. pylori* may be as low as 55%.[265] The best evidence for a causal association between *H. pylori* and ulcer disease comes from therapeutic trials. Persons treated with antibiotics in addition to acid inhibitory therapy have lower rates of ulcer relapse than persons treated with acid inhibitory therapy alone.[108,126] Moreover, if the antimicrobial agents used do not successfully eradicate *H. pylori* infection, there is no benefit over acid inhibition alone.

Only a minority (approximately 20%) of infected persons will develop duodenal ulcer within a 10 years of diagnosis.[293] Factors that determine infection outcome include bacterial characteristics, host characteristics, and the environment. Strains containing the Cag PAI[231] and the s1m1 allele of *vacA* have higher-than-average risk.[18] Also at higher risk are individuals with genotypes of the cytokines TNF-α and IL-1β that induce greater inflammatory responses. Environmental cofactors such as cigarette and alcohol use, use of NSAIDs, male gender, and blood group O and other hereditary factors have also been associated with magnified ulcer risk.[295]

8.1.4. Gastric Adenocarcinoma. It has been estimated that *H. pylori* infection may be responsible for 5.5% of all cancers worldwide.[241] In 1994, the World Health Organization (WHO) declared *H. pylori* infection to be an important cause of gastric adenocarcinoma.[135] Support for this declaration came from two types of data: studies of the natural history of gastric cancer and epidemiological studies of the association of *H. pylori* infection and cancer. Since gastric cancer usually occurs in the setting of chronic superficial gastritis, a relationship with *H. pylori* can be deduced. The most compelling data linking *H. pylori* infection with cancer, however, came from epidemiological investigations. Several types of studies have been done including studies paralleling the epidemiological features of *H. pylori* infection and gastric cancer, case–control studies of patients with cancer, and nested case–control studies within large cohort populations followed, prospectively, for cancer. These studies show that (1) gastric cancer occurs at an increased rate in regions with high prevalence of *H. pylori* infection, (2) cancer patients have higher rates of *H. pylori* infection than controls, and (3) *H. pylori* infection precedes cancer by many years (1993).[230,246] In a large prospective study conducted in Japan, no uninfected persons developed cancer within 8 years, whereas 2.9% of infected hosts did.[331]

Although non-randomized, prospective studies demonstrate cancer protection with *H. pylori* eradication,[112,309] definitive randomized trials are still lacking. The single randomized, placebo-controlled trial completed to date showed decreased cancer incidence following *H. pylori* eradication only in the small subgroup of individuals without preneoplastic conditions; in the total population treated, significant protection in the 7 years following *H. pylori* eradication was not observed.[350] Because of the relatively short follow-up and smallish sample size in this study, further trials are warranted. Despite the absence of conclusive data, many investigators maintain that—based on the wealth of information collected to date—*H. pylori eradication* will ultimately prove effective in preventing the majority of gastric adenocarcinomas.

As with ulcer disease, gastric adenocarcinoma only occurs in a small minority of infected subjects. Again, bacterial factors (e.g., the presence of the pathogenicity island), host factors (e.g., cytokine genotype), and diet (low antioxidants, high salt and nitrate) play a contributing role.

8.1.5. Gastric Lymphoma. Gastric lymphoma is the rarest of the diseases associated with *H. pylori* infection, with only seven cases per million population per year.[289] The normal stomach is not a lymphoid organ. Since *H. pylori* is the preeminent initiator of inflammation and lymphoid follicle formation in the stomach, it seems intuitive that it would be linked to lymphoma. Several cross-sectional and ecological studies have weakly supported this conjecture.[58,81] One somewhat stronger study demonstrated that *H. pylori* infection increased risk of later developing diffuse histiocytic gastric lymphomas 6-fold.[247] A particularly strong association of infection has been observed for mucosa-associated lymphoid tissue (MALT) lymphoma, a type of low-grade lymphoma that arises from lymphoid follicles.[311,351] The

remarkable feature of these MALT lymphomas is that 80% of tumors involving only the mucosa and submucosa completely regress if *H. pylori* infection is eradicated with antibiotics.[306] Recurrence of lymphoma in 5% is associated with both recurrent infection and t(11;18) rearrangements of the immunoglobulin heavy chain.[354]

Why a scattered few people with *H. pylori* develop these rare lymphomas is unknown. Some believe that infection may precipitate an autoimmune response that predisposes to malignancy.[114,136] Others hypothesize that *H. pylori* antigens themselves may drive lymphoproliferation.[247] It is hoped that understanding the role of *H. pylori* and lymphoma will shed light not only on the pathogenesis of gastric lymphomas but on other extranodal lymphomas as well.

8.1.6. Esophageal Diseases. As *H. pylori* prevalence has diminished over time, two diseases have been on the rise: gastroesophageal reflux disease (GERD) and esophageal adenocarcinoma. Both GERD and esophageal adenocarcinoma are thought to stem, at least in part, from acid damage to the distal esophagus. Without reflux (related to obesity and structural abnormalities of the stomach and esophagus) and without acid (related to the secretory capacity of the stomach), presumably, a large proportion of GERD and esophageal adenocarcinoma would not occur. The inverse temporal correlation between *H. pylori* and esophageal distal diseases, in conjunction with the hypochlorhydria that occurs in *H. pylori*-associated multifocal atrophic gastritis, has led investigators to postulate a protective role for the organism. To date, however, no consensus has been reached on this topic. A meta-analysis of the prevalence of *H. pylori* in individuals with and without GERD identified an overall protective effect.[56,268] Significant protection was not observed in all populations, however, but seemed to predominate in Asian populations.[268] Studies on *H. pylori* eradication and subsequent development of GERD have found widely varying results but no consistent deleterious effect.[56] Two prospective studies of esophageal malignancies, in contrast, have also shown a strong protective effect of *H. pylori.*[53,125] One of these two studies evaluated only adenocarcinomas[53] whereas the other found the effect predominantly in squamous cell carcinomas.[125] Retrospective studies have often, but not unanimously, supported these findings.[352,353,354] Thus, much is left to be learned before the deleterious effects of *H. pylori* can be balanced with its somewhat more hypothetical benefits.

8.2. Diagnosis

There is no consensus on who should be tested for *H. pylori.* In their only conference on the topic—now over a decade ago—Sthe National Institutes of Health recommended that diagnostic tests for *H. pylori* be conducted in (1) all persons with active gastric or duodenal ulcer and (2) persons on maintenance histamine antagonists for prevention of recurrent peptic ulcer disease (1994). Because ulcer disease cannot be distinguished from other causes of dyspepsia on clinical grounds,[217] this approach presupposes endoscopy. Instead, many primary care clinicians favor testing for *H. pylori* in dyspeptics who fail acid inhibitory therapy, treating those infected, and offering endoscopy only to those with no response to eradication therapy. Analyses have shown that this approach is cost-effective and spares endoscopy in many individuals.[79,80] In fact, a recent, large cohort study in UK indicates that treating and screening *all* individuals, regardless of symptoms, can be cost-effective in preventing dyspepsia and its related health care costs.[79] Unless this becomes the recommendation of professional groups,[79] dyspepsia in young adults will likely remain managed via the primary care strategy above, whereas in older adults who are at risk for malignancy, endoscopy remains the approach of choice. To prevent gastric cancer, consensus groups have also recommended screening individuals with a family history of the malignancy and those with prior gastric resection.[197]

For patients undergoing endoscopy, a rapid urease test is the diagnostic test of choice. This test is less expensive and more rapid than histological examination and/or culture. For adult patients in whom endoscopy is not necessary, a stool antigen or breath test is the best option, since both can be used to assess success of treatment. Serologic assays are best used for epidemiologic studies. They have high sensitivity for ongoing infection but titers respond slowly to eradication therapy and may not regress to negative.[48,143] Diagnostic tests have not been well assessed in children. High frequency of "transient" positive tests in young children are likely, in part, to reflect inconsistent performance of these assays.

9. Control and Prevention

9.1. General Concepts

Since the mechanisms of transmission of *H. pylori* are still uncertain, there are no current recommendations for control and prevention. Based on epidemiological studies indicating person-to-person transmission, it is likely that general improvements in hygiene standards such as hand washing and good household sanitation will decrease transmission.

9.2. Antibiotic and Chemotherapeutic Approaches to Disease Prevention

H. pylori is curable with antimicrobial therapy. The two most widely use regimens are 10 days of proton pump inhibitor triple therapy (a proton pump inhibitor plus clarithromycin and either amoxicillin or metronidazole; 80–90% cure) or 2 weeks of bismuth triple therapy (a combination of metronidazole, tetracycline, and bismuth; 70–90% cure). Unfortunately, antimicrobial resistance is on the rise.[209] Although in vitro resistance to amoxicillin and tetracycline remains virtually non-existent, resistances to metronidazole and clarithromycin occur at rates of 10–15% and 20–40%, respectively. In vitro clarithromycin resistance portends a 70% decrease of treatment efficacy whereas metronidazole resistance has a somewhat less clinical significance (25% decreased treatment efficacy). Eradication failures are treated with 2 weeks of four drugs (two antibiotics, a proton pump inhibitor, and bismuth), omitting clarithromycin if it was used in the first treatment course.

Whereas consensus has been achieved on antimicrobial treatment, controversy rages over whom to treat. At their only consensus conference on the subject, the National Institutes of Health in 1994 recommended treating only persons with active ulcer disease (gastric or duodenal) or persons on maintenance antihistamine therapy for recurrent ulcer disease.[40] The 2005 European consensus from Maastricht recommended additionally treating those with non-ulcer dyspepsia, gastric lymphoma, previous gastric resection or gastric cancer, atrophic gastritis, and gastric cancer in first-degree relatives. The Maastricht consensus group further recommended treatment in anyone who wishes it.[198] Still others maintain that eradicating *H. pylori* in all those infected, regardless of symptoms, is the correct approach and a recent clinical trial supports this view as potentially beneficial though not cost-effective.[170] As new information accumulates, it is likely that diagnosis and therapy will be offered to a wider segment of the population in order to prevent adverse outcomes of infection. For now, however, there are no recommendations for diagnostic testing and/or treatment in asymptomatic persons or persons with dyspepsia of unknown etiology, particularly in regions where *H. pylori* prevalence is relatively low (<20%).

9.3. Immunization

No vaccine is currently available to prevent or treat *H. pylori* infection although vaccine development is an area of considerable interest. Because the brisk, natural immune response neither eliminates infection nor prevents reinfection, one might feel skeptical that a vaccine could be efficacious. Despite this theoretical concern, however, several candidate vaccines are beginning to show promise in animal models.[200] With advancements in our understanding of the biology, new drug targets against essential metabolic genes are also an active area of research.[281] Were such a vaccine available, it is not yet known how it would optimally be used.

10. Unresolved Problems

Since 1982, an enormous amount of research has been conducted on *H. pylori* infection. In the last 5 years alone, over 6,000 manuscripts were published. Yet many of the critical issues concerning *H. pylori* remain to be understood.

Because acute infection almost always remains undiagnosed, the infectious inoculum has not been established. Moreover, it is unknown whether host factors affect the infectious inoculum or whether all uninfected persons are equally at risk. People who have been experimentally infected have always taken acid inhibitory therapy prior to ingesting the organism. Whether hypochlorhydria is actually required to permit infection to be established is not known. Neither symptoms of naturally acquired acute infection nor transmission route are firmly established. Although there is strong, circumstantial evidence for person-to-person transmission, it is debated whether waterborne transmission also occurs.

The interplay of *H. pylori* with other disease cofactors is undoubtedly complex and will take years, if not decades, to parse out. It is assumed that differences in outcome can be explained by a combination of bacterial factors (e.g., presence of cytotoxin), environmental factors (e.g., diet, cigarettes, etc.), host factors (e.g., cytokine genotype), and temporal factors (e.g., age at acquisition of infection). The lack of relevant animal models particularly hampers understanding of these processes.

From a public health perspective, there remains no good algorithm for disease prevention. Over half of the world's population is infected with *H. pylori*. In developing countries, *H. pylori* infection is almost universal. In developed countries, on the other hand, the incidence of infection has been rapidly decreasing without any specific antibacterial intervention. Adverse outcomes of *H. pylori* infection, such as gastric cancer and ulcer disease, are also on an impressive decline. Improvements in hygiene, diet, and other socioeconomic factors, rather than antibiotic use, probably account for this favorable slope. Should we be focusing on vaccine development for a disease that disappears with improvements in hygiene? Should we be screening and treating persons in

order to prevent *H. pylori*-related diseases? Should we be treating the families of infected persons in order to interrupt the cycle of reinfection? Does *H. pylori* have any benefits? Would widespread use of antibiotics to treat *H. pylori* produce more harm in terms of the overall burden of antimicrobial resistance than good? These very fundamental issues remain to be resolved.

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CHAPTER 19

Legionellosis

Roopal Patel, Matthew R. Moore, and Barry S. Fields

1. Introduction

Legionellosis is infection with bacteria of the genus *Legionella*. Legionellosis is primarily associated with two clinically and epidemiologically distinct syndromes: Legionnaires' disease, a potentially fatal form of pneumonia, and Pontiac fever, a self-limited, nonpneumonic illness (Table 1). At least 49 *Legionella* species and 71 serogroups have been identified, but one species, *Legionella pneumophila*, causes over 90% of Legionnaires' disease cases. The majority of *L. pneumophila* infections are caused by serogroup 1.[67] Legionellosis may occur in outbreaks or as sporadic cases (i.e., not part of a recognized outbreak). Although Legionnaires' disease outbreaks capture much media attention, the majority of reported cases are sporadic.[132] Less than 5% of cases are reported to public health authorities. Pontiac fever is generally recognized only when clusters of illness occur. Legionnaires' disease is an important cause of community-acquired and nosocomial pneumonia. At least 8,000–18,000 cases of Legionnaires' disease are hospitalized each year in the United States.[133]

2. Historical Background

Between July 21 and July 24, 1976, 4,400 visitors attended the 58th annual convention of the American Legion's Pennsylvania Department at the Bellevue-Stratford Hotel in Philadelphia. None could have known that events surrounding the meeting would be chronicled on the front pages of newspapers and in medical journals around the world. Between July 22 and August 3, 1976, dozens of conventioneers developed a mysterious febrile illness with pneumonia; no etiology was identified. During the investigation that followed, a total of 182 cases of the illness were identified among persons who had entered the Bellevue-Stratford between July 1 and August 18, 1976.[74] Many of the conventioneers became ill after returning home from the convention, yet illness was not identified among family members who had not attended the convention, indicating that it was not likely transmitted from person to person. Illness was reported for 39 additional persons who had no direct connection to either the convention or the hotel, except that all had been within one block of the Bellevue-Stratford at some time after July 1. This finding, together with the higher attack rate among convention delegates who had watched a parade from the sidewalk in front of the hotel, suggested airborne transmission of an agent in the environment. Altogether, 221 people became ill and 34 died of the illness, which became known as Legionnaires' disease. The cause of the outbreak remained unknown for 5 months despite intensive investigation, until early in 1977, when McDade and co-workers[136] recovered a fastidious, weakly gram-negative bacillus (subsequently named *Legionella pneumophila*) from guinea pigs inoculated with lung tissues of patients who had died during the outbreak. Paired acute- and convalescent-phase serum specimens from other ill conventioneers demonstrated rising levels of specific antibody to the organism, and high levels of antibody were present in over 90% of convalescent-phase specimens from ill conventioneers[7] The source of the organism at the Bellevue-Stratford was not identified.[72]

Serologic tests indicated that the newly identified organism was also the etiologic agent of earlier, unsolved outbreaks of respiratory illness and cases of sporadic pneumonia. Antibody tests performed on paired serum specimens that had been stored for several years at the Centers for Disease Control and Prevention (CDC) showed that the same

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Table 1. Clinical and Epidemiological Characteristics of the Two Forms of Legionellosis

Characteristic	Legionnaires' disease	Pontiac fever
Symptoms	Fever, cough, headache, myalgia, dyspnea, chest pain, diarrhea, delirium	Fever, headache, myalgia, cough, sore throat, chest tightness
Chest X ray	Pneumonia	Normal
Primary treatment	Erythromycin	No specific therapy
Attack rate in outbreaks	<5%	>95%
Incubation period	2–10 days	<48 hours
Mortality	5–30%	None

organism, or an antigenically similar one, had caused an outbreak of 81 cases of pneumonia with 14 deaths in 1965 at a psychiatric hospital in Washington, DC.[184] Outbreaks of pneumonia occurring as early as 1957 were associated with serological evidence of legionellosis.[148] Also, a "*Rickettsia*-like" organism that had been isolated in 1947, in guinea pigs inoculated with blood of a patient with a febrile illness, was subsequently shown to be *L. pneumophila*.[135] Other organisms that had been isolated before 1976, but only partially characterized, were identified as *Legionella* species. An organism isolated from guinea pigs injected with the blood of a febrile soldier at Fort Bragg, North Carolina, in 1943, was identified as *L. micdadei*,[182] and the same agent was described in 1979 as the cause of acute pneumonia in renal transplant recipients.[150] *L. pneumophila* was also identified as the etiologic agent of an outbreak with a high attack rate of a self-limited, nonpneumonic illness with fever (Pontiac fever) occurring in 1968 at a health department building in Southfield, MI.[77] Stored lung tissue from guinea pigs that had been exposed to air in the health department building and had developed pneumonia in 1968 yielded *L. pneumophila* a decade later.[107]

The environmental source and means of transmission of the organism were unknown until 1978 when *L. pneumophila* was recovered from various environmental specimens in conjunction with investigations of outbreaks that year.[141] Aerosolized water from cooling towers and evaporative condensers (heat rejection devices used in the air-conditioning systems of large buildings) was implicated as the source of dissemination of *L. pneumophila* in outbreaks of Legionnaires' disease in Memphis and Atlanta during 1978.[46,54] In 1980, Tobin and colleagues[186] isolated the same serogroup of *L. pneumophila* from two people who developed pneumonia after receiving renal transplants in an Oxford, England, hospital and from water taken from the shower in their common hospital room, indicating the role of potable water systems in the dissemination of *Legionella* species. Subsequent investigations conducted in the 1980s and 1990s further documented the role of cooling towers and potable water systems in the transmission of legionellosis, and dissemination of legionellae from other aerosol-producing devices have been reported (see Mechanism and Routes of Transmission below).

3. Methodology

3.1. Sources of Mortality Data

The gross and microscopic pathological findings in material obtained at autopsy from patients who died of Legionnaires' disease do not differ substantially from those found in cases of severe pneumonia caused by other bacteria.[155] Direct immunofluorescent antibody (DFA) staining has been widely used to identify the organisms in lung tissue postmortem.[43,155] Monoclonal DFA reagents are designed to detect only a single species or serogroup. Polyclonal reagents but are more likely to cross-react with bacteria that may colonize the respiratory tract, such as *Pseudomonas* spp.[183] Either method of DFA testing may be performed on formalin-fixed lung scrapings, paraffin-embedded tissue sections, or fresh lung tissue,[43,155] although polyclonal reagents may be preferable for tissues fixed for prolonged periods in formalin.[58,59] A *Legionella*-specific immunohistochemical stain developed by Centers for Disease Control and Prevention (CDC) has been successful in *Legionella* identification in formalin-fixed tissue[68] and the stain is routinely used by CDC's pathology laboratory in respiratory disease outbreak investigations. Culture and polymerase chain reaction (PCR) have also been used to identify the organism in lung tissue obtained at autopsy, but care must be taken to avoid false-positive results caused by contamination during specimen processing.[120] The exact role of legionellosis in causing death may be difficult to determine because other serious, life-threatening underlying illnesses are often present. Additionally, bacteria other than *Legionella* spp. occasionally may be isolated from the lungs of persons who have died of Legionnaires' disease.

Comprehensive, systematic, and reliable data on mortality due to Legionnaires' disease are unavailable. Although the National Center for Health Statistics (NCHS) receives copies of death certificates to tabulate national figures on deaths attributed to pneumonia and influenza based on International Classification of Diseases (ICD-9) codes, identification of a specific etiologic agent is lacking in the majority of cases. Data regarding the prevalence of Legionnaires' disease among patients who have died with pneumonia have

been systematically sought in one multi-city survey of fatal nosocomial pneumonia, in autopsy surveys of one or more hospitals in a city, and in conjunction with investigations of outbreaks.[44,179] Estimates of disease incidence from these studies are limited by small sample sizes and by nonrandom selection of hospitals surveyed. Review of autopsy material during epidemic investigations has been useful for defining the magnitude of the individual outbreaks. The case-fatality rate can be estimated by determining the proportion of fatal cases among those reported through surveillance for Legionnaires' disease; however, these estimates may be inaccurate because of underreporting of cases.[132] Additionally, the diagnosis may be confirmed more often among patients who die than those who survive because of more aggressive diagnostic testing among persons with severe pneumonia.

3.2. Sources of Morbidity Data

The limited data we have on the incidence and prevalence of Legionnaires' disease in the general population are based on findings from studies of patients with community-acquired pneumonia.[21,63,71,129,133,147,162,194] The most dependable data are from studies that are prospective and population based. The findings of these studies are affected by the criteria used to identify patients for laboratory testing and the type of laboratory tests used for case confirmation. Studies of incidence and prevalence of health care-associated legionellosis[28,100,101,131,142,157] are influenced by the same factors that affect studies of patients with community-acquired infection. Additionally, the incidence of health care-associated infection varies greatly from one institution to another because of the variation in hospital facilities and patients.

Surveillance for legionellosis is conducted with varying intensity in many countries. In the United States, Legionnaires' disease is a condition reportable to the Centers for Disease Control and Prevention (CDC) through the National Notifiable Disease Surveillance System (NNDSS). Reports through NNDSS from state health departments include only basic demographic information for cases of legionellosis. A supplementary paper-based surveillance system for Legionnaires' disease collects more detailed information including whether cases are travel, health care, and/or outbreak related. However, data from prospective studies of patients with pneumonia indicate that less than 5% of legionellosis cases are reported to CDC.[132,133] The Council of State and Territorial Epidemiologists (CSTE) recently revised their position statement for legionellosis surveillance. In addition to modifying the case definition, the position statement set goals for the timeline of case reporting to improve overall surveillance and to facilitate timely identification and the subsequent investigation of travel-related cases. In Europe, over 30 countries participate in the European surveillance scheme for travel-associated Legionnaires' disease. In addition to timely reporting to the European Working Group for *Legionella* Infections (EWGLI) of travel-related cases, each year the participating countries submit standardized case report forms that provide epidemiological and microbiologic information on the total number of travel-related and non-travel-related cases. EWGLI then reports an analysis of national and European aggregate trends.[103]

3.3. Surveys

The indirect immunofluorescent antibody (IFA) test has been used most widely in serological surveys, although other, less standardized methods, including hemagglutination, indirect hemagglutination, immune adherence, microagglutination test, and enzyme-linked immunosorbent assay (ELISA), have also been used. Differences in methods of preparing test antigens and possible cross-reactions with other organisms, especially at relatively low titers, make the interpretation of serological surveys difficult. Serological tests prepared from heat-killed antigens, the most widely used test in North America, may not be as specific as those using formalinized antigen, which are commonly used in Europe.[60,61] Rates of elevated IFA titers ($\geq$ 1:128) to *L. pneumophila* in the general population have ranged from 1 to 36%[60,61,87,177]; generally, the higher rates are observed when pooled antigens from several *L. pneumophila* serogroups are used for preparation of reagents and the lower rates are seen in studies using tests specific for *L. pneumophila* serogroup 1. The relatively high background rates of elevated titers indicate that the positive predictive value of a single titer for determining recent legionellosis is low. The frequency with which legionellosis can occur as an asymptomatic or subclinical infection has been estimated by testing paired serum specimens from exposed well persons in conjunction with outbreak investigations.[56]

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification. *Legionella* cannot be cultured by routine clinical bacteriologic techniques. Charcoal yeast extract agar is the base form for most media used to grow legionellae.[65] The medium used for the culture of legionellae has been improved several times, eventually resulting in the medium currently used, buffered charcoal yeast extract (BCYE) agar enriched with α-ketoglutarate with and without selective agents added.[57] BCYE agar plates are incubated at 37°C in an atmosphere of 2.5%

CO_2. Legionellae require 3–14 days to grow. *Legionella*-like colonies are identified using a dissecting microscope and light source to detect colonies resembling legionellae. After approximately 4 days of incubation, these colonies are 2–4 mm in diameter, convex, and round with entire edges. The colonies are usually white in the center bordered with blue, purple, green, or red iridescence. Colonies resembling legionellae can be presumptively identified on the basis of their requirement for the amino acid L-cysteine by subculture on blood agar or BCYE agar without L-cysteine. Subcultured colonies that grow on BCYE agar, but not on blood agar or BCYE without L-cysteine, are presumed to be legionellae. Definitive identification is usually accomplished by using a direct fluorescent antibody (DFA) or slide agglutination test with specific antisera.

Culture remains the gold standard for diagnosing legionellosis and for identifying the organism in the environment. Based upon culture of serologically positive (4-fold rise) patients, the sensitivity is near 60% and the specificity is near 100%.[58,59,123] However, a number of factors limit the sensitivity of culture. Laboratories experienced in the isolation of legionellae are more likely to recover the organism. A survey by the College of American Pathologists indicated that as many as two thirds of clinical microbiology laboratories in the United States are unable to grow a pure and heavy culture of *L. pneumophila*.[60,61] Moreover, to improve the specificity of the diagnosis of pneumonia caused by pyogenic bacteria, a common practice in hospital laboratories is to reject sputum specimens containing many squamous epithelial cells or few polymorphonuclear leukocytes; however, some patients with Legionnaires' disease produce little or nonpurulent sputum, and sputum specimens that would be routinely discarded may contain culturable legionellae.[95] The bacteria survive poorly in respiratory secretions, and immediate culture of these specimens is critical.[67] The reported sensitivity of culture ranges widely from 20 to 80%, reflecting the perishable nature of the specimens and the expertise required.[158]

Legionellae can be isolated from a number of specimens, including blood, lung tissue, lung biopsy specimens, respiratory secretions (sputum, bronchial alveolar lavage (BAL) bronchial aspirates), and stool. Respiratory secretions are considered the specimens of choice. Sputum is considered less suitable than other respiratory secretions, especially in early disease when few patients have a productive cough.[123] When culturing sputum it is best to pretreat the sample by either acidification or heat. *Legionella* has also been cultured from a number of extrapulmonary sites such as bone marrow, prosthetic heart valves, and sternal wounds.[55,60,61] Several guidelines give detailed methods for the isolation of *Legionella* from clinical samples.[55,178,188]

3.4.2. Serological Diagnostic Methods. Although serological tests are widely used for the diagnosis of legionellosis, these tests have a number of important and often under-recognized limitations. Even in cases of culture-confirmed Legionnaires' disease, a 4-fold rise in antibody by IFA can be documented for only 70–80% of patients, and seroconversion following legionellosis may not occur for up to 2 months following the onset of illness.[60,61] Reagents that detect all major immunoglobulin classes (IgG, IgM, and IgA) are generally used. Testing for IgM alone has not proved useful for the diagnosis of legionellosis because IgM often increases concomitantly with IgG and may remain elevated for months or years after infection.[33] A single elevated antibody titer does not confirm the diagnosis of legionellosis. In a large study of patients hospitalized with community-acquired pneumonia in Ohio, a single titer of $\geq$ 1:256 did not distinguish between Legionnaires' disease and pneumonia due to other agents: only 10% of 68 patients with legionellosis confirmed by culture or 4-fold rise in antibody had acute-phase titers of $\geq$256 compared with 6% of 636 patients with pneumonia caused by other agents.[153] Current recommendations suggest the use of *L. pneumophila* serogroup 1 antigen only and the avoidance of polyvalent antigen pools. It is extremely difficult to make a diagnosis based on results of IFA tests with polyvalent pools.

Microscopic examination of specimens using DFA staining can be used to detect legionellae in lung tissue (from autopsy or biopsy) and in respiratory secretions. The sensitivity of DFA testing of respiratory secretions for the diagnosis of Legionnaires' disease has ranged from 25 to 75%, and specificity is >95%.[60,61] While DFA provides a rapid method of identifying *Legionella* spp., immunofluorescent microscopy is technically demanding and should be performed only by laboratory personnel experienced in the procedure. The specificity of polyvalent DFA reagents is probably lower than that of monoclonal reagents, leading some clinical microbiologists to recommend against routine use of polyvalent reagents.[60,61] Legionellae can be detected in respiratory secretions by DFA for several days after the start of antimicrobial therapy.

3.4.3. Urine Antigen Detection. Urinary antigen testing has led to the recognition of outbreaks of Legionnaires' disease and allowed for rapid public health response.[62,68,117] In addition, urine antigen testing permits early diagnosis and initiation of appropriate antibiotic therapy. The capture antibody used in the majority of these assays is considered to be specific for *L. pneumophila* serogroup 1. Therefore, even though the majority of human diseases are associated with *L. pneumophila* serogroup 1,

total dependence upon this diagnostic assay may miss as many as 40% of the cases of legionellosis. *Legionella* antigen present in urine specimens appears to degrade upon prolonged storage.[156] There is a single report of a false-positive urinary antigen assay on a specimen obtained from a renal transplant patient who had received rabbit serum with antibodies to thymocytes.[48]

Changes in the use of diagnostic tests for Legionnaires' disease may have had an impact on the number of reported cases, the reported mortality, and the distribution of serotypes. In the United States, cases diagnosed by culture have declined while cases diagnosed by urine antigen have increased. Since urine antigen testing allows for earlier diagnosis, this may have allowed for more appropriate antimicrobial therapy and improved the outcome of Legionnaires' disease. However, since urine antigen testing primarily detects serogroup 1 infections, cases of Legionnaires' disease caused by other serogroups and species may have been missed.[14]

Shortly after the outbreak of Legionnaires' disease at the American Legion Convention in Philadelphia, investigators reported the ability to detect antigen in the urine of serologically confirmed Legionnaires' disease patients by ELISA.[18,185] The antigen detected is a component of the lipopolysaccharide portion of *Legionella* cell wall and is heat stable.[111,191] Antigens are generally detectable in urine within a few days of illness onset and can remain so for several weeks after initiation of appropriate antimicrobial therapy.[110] These studies have shown that antigen is excreted as soon as 3 days after the onset of symptoms and can persist for >300 days. Antigens detected in 88% of patients tested during days 1–3, 80% during days 4–7, 89% during days 8–14, and 100% after day 14 were positive.

Currently, there are three commercially available ELISA urinary antigen tests: Wampole (formerly distributed by Binax, Inc.), Cranbury, New Jersey; Trinity Biotech, Ireland; and Biotest AG, Dreieich, Germany. Two of these tests (Wampole and Trinity Biotech) are intended to be specific for *L. pneumophila* serogroup 1 and are not likely to detect the 20–30% of Legionnaires' disease cases that are caused by other serogroups and species.[123] The Biotest ELISA is intended to detect antigen of other *L. pneumophila* serogroups and *Legionella* species.

The most recent method for detecting antigenuria is the immunochromatographic (ICT) membrane assay. The ICT assay is similar to a home pregnancy test and is commercially available (NOW *Legionella* urinary antigen test, Binax, Inc., Portland, ME). The test is simple to perform, does not require special laboratory equipment, and results can be obtained within 15 minutes. A comparison of the Binax NOW with the Binax EIA showed 98% overall agreement between the two tests and a specificity of 100%.[52] The authors suggested using concentrated urine samples to increase test specificity. Another study determined that the sensitivity of the ICT assay for *L. pneumophila* serogroup 1 was 80% and the specificity 97%.[86]

3.4.4. Nucleic Acid Detection. Polymerase chain reaction (PCR) represents one of the few diagnostic tests with the potential to detect infections caused by all of the known species of *Legionella*. Since most rapid tests only detect infections due to *L. pneumophila* serogroup 1, an accurate PCR test would greatly enhance the ability to diagnose these infections. Various PCR tests have been developed for legionellae targeting several genes.[67] A limited number of laboratories test for legionellae by PCR at this time. The few studies which have been conducted indicated that the PCR detection of legionellae infections has a moderate sensitivity and a high specificity. Legionellae DNA has been detected in respiratory secretions such as BAL, pharyngeal swabs, nasopharyngeal swabs, peripheral blood mononuclear cells, urine, and serum.[67]

Numerous studies have reported development of real-time PCR combined with the use of a hybridization probe to confirm the product identity.[11,78] These methods reduce the risk of cross-contamination, the time to process samples, and eliminate the need for sample analysis after PCR. Limited studies and preliminary data suggest that PCR may add to the diagnostic repertoire, but this procedure may lack the sensitivity and specificity to provide anything other than supplemental data for the clinician.

3.4.5. Biological Characteristics of the Organism. Bacteria of the genus *Legionella* are gram-negative, aerobic, rod-shaped bacteria. The bacterial cells are 0.3–0.9 by 1–20 μm and motile, with one or more polar or lateral flagella.[29] Currently, there are 49 species comprising 71 distinct serogroups in the genus *Legionella*.[15,67,149] One species, *L. pneumophila*, causes approximately 90% of all reported cases of legionellosis in the United States. This figure may be inflated as most diagnostic tests are specific to *L. pneumophila*. Approximately half of the 49 species of legionellae have been associated with human disease and it is likely that many of the rest can cause disease under appropriate conditions.

Legionellae use amino acids as their carbon and energy sources and do not oxidize or ferment carbohydrates.[66] The bacteria can be grown in the laboratory on buffered charcoal yeast extract (BCYE) agar. This is a complex medium supplemented with soluble iron and the amino acid cysteine. These fastidious growth requirements are reflect the bacteria's existence as a facultative intracellular parasite of eukaryotic cells. Legionellae multiply intracellularly

in freshwater protozoa in the environment and in monocytes and alveolar macrophages of humans.[66] Legionellae are readily phagocytized by alveolar macrophages and monocytes and are able to multiply intracellularly within specialized phagosomes.[94] Large numbers of organisms can be visualized and recovered from the lungs of patients with Legionnaires' disease; however, the organism does not appear to asymptomatically colonize the respiratory tract.[30] Therefore, any *Legionella* species recovered from respiratory secretions should be viewed as a likely pathogen. Infection of both human phagocytic cells and freshwater protozoa is accomplished utilizing a type IVB secretion system composed of 25 genes.[166] This type IV secretion system delivers substrates which allow the bacteria subvert host cell processes for its own propagation.

Water is the major reservoir for legionellae, and the bacteria are found in freshwater environments worldwide.[70] Legionellae have been detected in as many as 40% of freshwater environments by culture and in up to 80% of freshwater sites tested by PCR.[67] A single exception to this observation is *L. longbeachae*, a frequent isolate from potting soil.[175] This species is the leading cause of legionellosis in Australia and occurs in gardeners and those exposed to commercial potting soil.[161] The first US cases of *L. longbeachae* infection associated with potting soil were reported in 2000.[39] *L. pneumophila* multiplies at temperatures between 25 and 42°C, with an optimal growth temperature of 35°C.[106] Most cases of legionellosis can be traced to man-made aquatic environments where the water temperature is higher than ambient temperature. Thermally altered aquatic environments can shift the balance between protozoa and bacteria, resulting in rapid multiplication of legionellae, which can translate into human disease.

4. Descriptive Epidemiology

4.1. Incidence and Prevalence

Surveillance for legionellosis does not yield an accurate measure of incidence because many cases are not diagnosed or reported. Furthermore, only a few population-based studies have assessed the incidence of legionellosis. In a population-based study of all adults hospitalized during 1991 with pneumonia in two counties in Ohio, the annual incidence of Legionnaires' disease was 7.0 cases per 100,000 adults.[133] After projecting the age- and race-specific incidence to the US population, the study estimated that 8,000–18,000 cases of Legionnaires' disease are hospitalized annually. This estimate is much greater than the number of cases reported to the CDC through the NNDSS each year which ranges from 1,200 to 2,000 cases.[41] The difference in the number of reported cases and estimated incidence may be due to several factors. Cases may be diagnosed but not reported to the state public health department or from the state to CDC. Or more often, cases may not be diagnosed because LD testing was not performed. In a study of patients participating in a prepaid health plan that covered 10% of the population in Seattle, Washington, 5 (1%) of 500 patients with community-acquired pneumonia (84% treated as outpatients) evaluated between 1963 and 1975 had legionellosis confirmed by a 4-fold rise in antibody titer.[71] Based on these findings, the annual incidence of legionellosis was estimated to be 12 cases per 100,000 persons (95% confidence interval: 4–28 per 100,000).[71] A much lower incidence was observed in a 1-year, community-based study in Nottingham, England (population approximately 70,000): Of 251 patients with pneumonia (78% managed without hospitalization), only 1 had legionellosis confirmed by serological testing, for an annual incidence of 1.4 cases per 100,000 persons.[194] A more recent prospective study in Barcelona, Spain, showed similar results (3.3 cases per 100,000 persons),[5] again lower than the incidence seen from studies in the United States. Results from population-based studies that focus on outpatients with community-acquired pneumonia underestimate the true incidence of Legionnaires' disease since the majority of LD infections are severe enough to require hospitalization.

The overall proportion of hospital-acquired pneumonia due to Legionnaires' disease is not known, although the percentage at individual hospitals has ranged from 0 to nearly 50%.[100,101,131,142,157,162] Of the cases reported to the CDC through the supplementary paper-based system, 35% are considered to be health care associated.[14] Surveillance data from the European Working Group for *Legionella* infections from 28 to 32 countries in 2000–2002 found that 8.6% of the total cases were health care associated.[103]

No data are available on the incidence or prevalence of Pontiac fever. Surveillance for Pontiac fever, although a part of NNDSS, is difficult because symptoms are often mild and nonspecific, and no rapid tests are available to confirm the diagnosis. Most reported Pontiac fever cases are outbreak related. Sporadic cases, if they occur, are not identified and little is know about them.

4.2. Epidemic Behavior and Contagiousness

Although 80–90% of legionellosis cases reported through surveillance in the United States and the United Kingdom are sporadic,[132] Legionnaires' disease can occur in explosive, common source outbreaks. Risk factors for

legionellosis have been identified primarily from epidemiologic investigations conducted in outbreak settings. Ongoing or recurrent outbreaks occurring over a period of years have been associated with exposure to colonized water systems in hotels and hospitals.[13,19,51] During outbreaks of Legionnaires' disease, attack rates among those exposed to the source of the organism are generally less than 5%, while the attack rate for Pontiac fever is much higher, up to 95% of exposed persons become ill.[91] In outbreaks of legionellosis, the risk of illness is often associated with the amount of time spent in the area of apparent exposure and, for Legionnaires' disease, but not Pontiac fever, the level of host susceptibility.

Although the organism is present in the respiratory secretions of patients with Legionnaires' disease, person-to-person transmission has not been documented. To assess the possibility of person-to-person transmission during the 1976 outbreak in Philadelphia, interviews were conducted with 193 household contacts of persons with Legionnaires' disease; no cases of illness suggestive of secondary spread during the 3 weeks following exposure were identified.[74] Similar interviews were conducted with household and hospital contacts of 27 patients during the investigation of an outbreak in Kingsport, Tennessee, in 1977; no evidence of person-to-person transmission was found.[53] Yu and colleagues found no instances of pneumonia or seroconversion to *L. pneumophila* among 17 household contacts of patients with community-acquired Legionnaires' disease or among 15 hospital roommates of patients with nosocomial infection.[196] There was no evidence of transmission of *L. pneumophila* from symptomatic, experimentally infected guinea pigs to uninfected animals in adjacent cages located <1 in. away.[105] One investigation found a higher prevalence of unexplained febrile respiratory illnesses and elevated antibody titers to *L. pneumophila* among hospital personnel in contact with Legionnaires' disease patients compared with health care workers without such exposure,[164] but these findings have not been reproduced. The elevated antibody levels among contacts of persons with legionellosis may reflect common exposure to an environmental source of the organism rather than person-to-person transmission, and the higher prevalence of self-reported respiratory illnesses among persons also reporting exposure to infected patients may be due to recall bias.

4.3. Geographic Distribution

Legionellosis occurs worldwide. Although most surveillance data are reported by countries in temperate regions, cases of Legionnaires' disease have been identified in tropical countries when diagnostic testing has been performed.[2,119] In the United States, the incidence of reported Legionnaires' disease cases varies greatly among the states, with higher rates observed in some northern states.[132] The observed regional variation in incidence may be due to dissimilar diagnostic and reporting practices or may reflect actual differences in incidence resulting from disparate ecological or epidemiological factors.

4.4. Seasonal Distribution

Rates of reported Legionnaires' disease cases vary seasonally, with the highest rates occurring during the summer months[132]; outbreaks of Legionnaires' disease associated with cooling towers often have occurred during the summer and fall months.[17] Nonetheless, during a prospective, population-based study of pneumonia in Ohio, where diagnostic tests for legionellosis were systematically obtained, cases of Legionnaires' disease were identified year-round without an excess of cases in the summer.[133] Thus, the reported seasonality of Legionnaires' disease could reflect an ascertainment bias, i.e., clinicians may suspect and order tests for legionellosis more frequently during the summer on the basis of early reports of Legionnaires' disease as a predominantly summer disease.[133]

4.5. Age

In the 1976 outbreak in Philadelphia and in many subsequent Legionnaires' disease outbreaks, attack rates increased progressively with increasing age.[74] The incidence of Legionnaires' disease identified through surveillance is highest among persons 70–79 years of age with a median age of 60 years.[14] The association between illness and advanced age may be confounded by the high prevalence of medical conditions that predispose to Legionnaires' disease among the elderly. Compared with adults, Legionnaires' disease appears to be an uncommon cause of pneumonia among children. Most reported cases of Legionnaires' disease in children were nosocomial infections that occurred in patients who had underlying immunosuppressive conditions.[34] However, seroconversion to *L. pneumophila* without respiratory disease is not uncommon during early childhood.[6] It is not clear whether these asymptomatic seroconversions among young children reflect frequent exposure to the organism or infection with serologically cross-reacting organisms.

Most information regarding the demographics of cases of Pontiac fever comes from outbreak investigations. In the

largest reported Pontiac fever outbreak in the past 10 years, the mean age of the cases was 37 years but the range was 4–76 years.[102]

4.6. Sex

Legionnaires' disease occurs more often among men than women.[132] It has not been determined whether this is the result of greater susceptibility, higher prevalence of certain predisposing conditions (e.g., cigarette smoking, chronic lung diseases), more diagnostic testing, or increased risk of exposure. Serosurveys have generally not demonstrated differences in prevalence of elevated titers to *L. pneumophila* among men and women,[154,177] although one study found a greater prevalence of reciprocal titers $\geq$128 among men aged 40 years and older compared with women in the same age range.[87] In outbreaks of Pontiac fever, attack rates among exposed males and females are approximately equal.

4.7. Race

During outbreaks, no consistent association between race and risk of legionellosis has been observed. One study found that the risk of community-acquired Legionnaires' disease was 14-fold higher among blacks than whites,[176] but among persons with Legionnaires' disease reported through surveillance during the 1980s and 1990s, there was no difference in the rate of disease among the races.[14]

4.8. Occupation

Outbreaks of legionellosis have occurred in a variety of workplace settings, including industrial plants[36,76,88] and office buildings.[45,75] Additionally, employees working in hospitals where transmission of Legionnaires' disease was occurring have become infected.[84,108] The relative importance of workplace exposure as a setting for transmission of non-outbreak-related legionellosis is unknown.

No particular occupation has been consistently identified as posing an increased risk of legionellosis. Construction workers were found to be at higher risk of sporadic Legionnaires' disease in one case–control study,[176] but a serological survey did not show a higher prevalence of elevated antibody titers to *L. pneumophila* among construction and other outdoor workers compared with white-collar personnel working indoors.[173] A survey comparing air-conditioning maintenance personnel with workers for the same company who had no direct exposure to cooling towers demonstrated no differences in history of pneumonia or elevated titers to *L. pneumophila* serogroup 1.[81] While one study found a higher prevalence of antibody to *L. pneumophila* among workers with greater exposure to colonized power plant cooling towers,[32] another study failed to demonstrate such a difference.[50] Asymptomatic maintenance employees who worked on air-conditioning cooling towers associated with an outbreak of Legionnaires' disease were more likely to have serological evidence of infection compared to co-workers with less exposure.[109]

4.9. Occurrence in Special Settings

4.9.1. Health Care-Associated Infection. Legionnaires' disease is an important and often under-diagnosed cause of health care-associated pneumonia. Hospitals provide a unique setting for the increased transmission of Legionnaires' disease because complex plumbing systems favor amplification of *Legionella* and large numbers of persons with underlying medical conditions may be exposed to the organism. Numerous outbreaks of health care-associated disease have been reported,[7,10,19,23,26,28,35,51,54,77,83,84,100,101,104,109,134,137,142,146,169,184,186,187] and exposures to aerosols generated by cooling towers, showers, faucets, and respiratory therapy equipment with contaminated potable water have been implicated. Aspiration of *Legionella*, especially among patients with nasogastric tubes or impaired swallowing reflexes, likely occurs. Transmission within hospitals with colonized hot water systems can occur intermittently for years.[19,51,114] In one hospital in Arizona, after three possible nosocomial cases were identified in a 3-month period, increased retrospective and prospective surveillance found 25 cases in over a 17-year period. Environmental studies implicated chronic colonization of the hospital potable water system as the source of these cases.[114]

The risk of acquiring Legionnaires' disease during hospitalization is influenced by a number of factors, including the type and intensity of exposure and the health status of the exposed.[41] Specific risk factors for nosocomial Legionnaires' disease include immunosuppressive medication (e.g., cytotoxic chemotherapy, corticosteroids), endotracheal intubation, general anesthesia, placement of a nasogastric tube, and occupying a hospital room near an aerosol source.[7,23,26,35,83,84,142] Showering was associated with nosocomial legionellosis in one study,[26] while another found showering to be significantly less common among patients with hospital-acquired infection.[23] The lack of consistent association with showering likely reflects different modes of transmission in various hospital settings.

4.9.2. Disease among Travelers. A history of recent travel is associated with approximately 21% of Legionnaires' disease cases in the United States[14] and 30% from European surveillance[103] are associated with recent travel. In a case–control study of sporadic community-acquired Legionnaires' disease in the United States, case-patients were more likely to have traveled during the 2 weeks before illness onset than were their acquaintances.[176] Outbreaks of legionellosis have been identified among guests at hotels and resorts and among passengers on cruise ships.[13,42,74,80,90,98,125,138,165] Outbreaks of Legionnaires' disease among travelers are under-recognized. Hotel guests and cruise ship passengers exposed during travel often return home before becoming ill; unless several members of the same travel party become ill, the clustering of cases and the association with travel may not be recognized. Pontiac fever outbreaks related to travel may be detected more readily because of the higher attack rate and shorter incubation period.

4.10. Socioeconomic Factors

Although socioeconomic status may be linked with risk factors for legionellosis (e.g., smoking, travel, underlying illness), a direct association between socioeconomic status and the risk of legionellosis has not been identified.

4.11. Other Factors

4.11.1. Cigarette Smoking. Cigarette smokers have a 2- to 4-fold increased risk of developing Legionnaires' disease than do nonsmokers, and the risk is greatest among more frequent smokers.[132,176] Alveolar macrophages harvested from healthy smokers have diminished resistance to intracellular replication of *L. pneumophila* compared with those from nonsmokers.[97] Cigarette smoking has not been associated with Pontiac fever.

4.11.2. Alcohol Consumption. Compared with cigarette smoking, alcohol consumption has been less consistently associated with Legionnaires' disease, and the association may be confounded by the higher prevalence of smoking among those who drink alcohol heavily.[96] After controlling for smoking in multivariate analysis, one study of sporadic, community-acquired disease demonstrated an increased risk of infection among persons who consumed three or more alcohol-containing drinks per day.[176]

4.11.3. Underlying Medical Conditions. The risk of acquiring Legionnaires' disease is over 20-fold greater for persons with hematologic malignancies or with end-stage renal disease than for the general population.[132] Risk of disease is also heightened for persons with diabetes mellitus or lung cancer and for patients receiving corticosteroids.[7,35,83,132,176] Legionnaires' disease is increasingly recognized in persons with acquired immunodeficiency syndrome (AIDS).[22] Results from a small number of cases reported through surveillance showed that the incidence of Legionnaires' disease among persons with AIDS is more than 40 times that of the general population.[132] However, in the largest series of Legionnaires' disease in patients with HIV reported, no statistically significant difference in outcome was found between patients with and without HIV, although there was a trend toward a worse outcome in the HIV-positive patients.[152] Pontiac fever does not have a predilection for persons with underlying illness.

5. Mechanism and Routes of Transmission

Inhalation of legionellae in aerosolized droplet nuclei is the primary means of transmission for legionellosis.[24] These aerosolized droplets must be of a respirable size (1–5 μm). A number of devices have been implicated as sources of aerosol transmission of legionellae (Table 2). These sources are associated with both potable and non-potable water. Sources producing aerosols of contaminated potable water include showers, faucets, and respiratory therapy equipment.[26,134] Sources using non-potable water

Table 2. Aerosol Sources for Transmission of Legionellosis Identified During Outbreak Investigations

Clinical syndrome	Aerosol source	Selected references
Legionnaires' disease	Cooling tower	(54, 12, 85, 109, 77, 1, 37)
	Evaporative condenser	(46, 26)
	Showers	(186, 84, 87)
	Faucets	(27)
	Respiratory therapy equipment	(7, 134)
	Portable room humidifier	(7)
	Whirlpool spa	(190, 98, 38)
	Decorative fountain	(90)
	Ultrasonic mist machine	(122)
Pontiac fever	Cooling tower	(74)
	Evaporative condenser	(107)
	Whirlpool spa	(174, 127, 80, 138)
	Decorative fountain	(90)
	Industrial metal work	(88, 76)
	Industrial cleaning processes	(73, 36)

include cooling towers, heated spas, decorative fountains, humidifiers, ultrasonic mist machines, and industrial manufacturing systems.[49,76,82,102,122] The role of home humidifiers in sporadic cases of Legionnaires' disease is unknown. Ducts and vents of air-conditioning systems can be conduits for passage of aerosol-containing *Legionella* organisms from nearby contaminated cooling towers. Most studies have indicated that aerosol from contaminated cooling towers can transmit disease within a limited range (<200 m); however, in certain circumstances, cooling towers may transmit *Legionella* to persons over longer distances.[1] Air-conditioning systems based on direct exchange of heat from refrigerant to air, without use of water evaporation (such as window and most other home air-conditioning units and automobile air conditioners), are not capable of transmitting disease. Legionellosis resulting from aspiration of upper respiratory secretions or gastric contents may occur among certain hospitalized patients.[100,101] Transmission of extrapulmonary *L. dumoffii* infection by direct inoculation of surgical wounds with colonized tap water during bathing or dressing changes has been reported.[121] Meaningful identification of sources of transmission requires a multidisciplinary approach including epidemiology, molecular epidemiology, and microbiologic techniques such as water and, occasionally, air sampling.

6. Pathogenesis and Immunity

The incubation period for Legionnaires' disease is generally 2–10 days from exposure until onset of pneumonia symptoms,[91] although incubation periods of 14 days or longer have been reported.[49,74] Inhaled organisms that reach the terminal alveoli are engulfed by alveolar macrophages and reside within specialized phagosomes that do not fuse with lysosomes, permitting the organism to escape intracellular killing.[92] Animal studies indicate that *L. pneumophila* replicates rapidly within 24 hours after infection[47]; large numbers of organisms are released from infected macrophages and, in turn, enter other phagocytes. Legionellae also appear to be capable of reproducing within alveolar epithelial cells, but the significance of this observation is not known.[139] An influx of polymorphonuclear leukocytes into the lungs occurs during the initial 3 days of infection, followed by an infiltration of mononuclear phagocytes.[47] Viable organisms are cleared from the lungs of surviving animals within 11 days of infection, and clearance coincides with the appearance of antibodies to *Legionella* in serum and alveolar lavage fluid.[47]

The gross pathological findings in Legionnaires' disease include heavy, congested lungs with consolidation distributed evenly through the lung parenchyma with no predilection for any specific lobe.[20,192] Consolidation is usually delineated by the lobular septa. In some cases, consolidation is distinctly nodular and may be mistaken for tumor nodules. Cut surfaces of involved portions of lung appear moist and airless. Although pulmonary cavities are rarely identified radiographically with Legionnaires' disease, multiple small macroscopic lung abscesses are observed in approximately 20% of fatal cases. Overtly hemorrhagic pulmonary parenchyma and empyema are unusual.

Microscopically, there is an acute fibropurulent pneumonia with neutrophils, macrophages, erythrocytes, and fibrin that fill the alveoli and respiratory bronchioles; these findings do not differ substantially from those of bronchopneumonia caused by other bacteria.[19,157] Superimposed features of diffuse alveolar damage, such as hyaline membranes, interstitial inflammation, and pneumocyte regeneration, may be present in severe cases. Although the cellular infiltrate is commonly necrotic and small abscess formation may occur, the underlying pulmonary structure remains intact. Legionellae are difficult to identify by Gram's stain, but will stain nonspecifically with Giemsa, Warthin–Starry, and Dieterle techniques.[19] Bacilli are commonly seen in clusters within macrophages. *L. micdadei* and *L. cincinnatiensis* in clinical specimens may stain with acid-fast techniques (e.g., Kinyoun stain), but will appear morphologically distinct from mycobacteria.[89,99,193]

Bacteremia may accompany Legionnaires' disease,[9] but whether symptoms referable to the gastrointestinal tract, kidneys, central nervous system, and muscle result from direct bacterial invasion (which has rarely been demonstrated in extrathoracic organs), toxins, or other indirect effects is not known. No consistent histopathologic changes have been demonstrated outside the lungs.

Cell-mediated immunity is important in host defense against legionellae, which multiply in human blood monocytes and alveolar macrophages.[92,145] Three different types of cellular responses contributing to immunity have been studied.[93] First, human monocytes and alveolar macrophages activated by lymphokines inhibit intracellular multiplication of *L. pneumophila*. Second, activated polymorphonuclear leukocytes have an enhanced capacity to kill the organism, although several days are required for killing, raising some questions about the significance of this immune mechanism. Third, cytotoxic lymphocytes may be capable of lysing infected macrophages, although evidence supporting this mechanism has not been reproduced.[93] Moreover, patients receiving medications that impair cell-mediated immunity are at increased risk of Legionnaires' disease. These observations suggest that cell-mediated immunity is the primary host defense against legionellosis, while humoral

immunity plays a secondary role. Anti-*L. pneumophila* antibody promotes phagocytosis of the organism by alveolar macrophages but does not promote intracellular killing.[145]

The level of protection against reinfection among humans after legionellosis is poorly understood. Guinea pigs exposed to sublethal doses of *L. pneumophila* in aerosols developed strong humoral and cell-mediated immune responses, and these animals survived subsequent challenge with large doses of aerosolized *L. pneumophila*, which killed control animals that were not previously exposed.[28]

Pontiac fever usually has an incubation period of less than 48 hours after legionellae are inhaled.[79] The pathogenesis of this syndrome is not understood, in part because the disease is not fatal and no pathological material has been examined. It is also unknown whether proliferation of bacteria is necessary to produce the syndrome and, if it is, where the proliferation occurs. Hypersensitivity to inhaled amoebae that are infected with legionellae has been proposed as a possible cause of Pontiac fever.[36,160]

7. Patterns of Host Response

7.1. Clinical Features

Legionnaires' disease is a type of pneumonia that may also involve other organ systems. Clinical features may include fever, cough, headache, myalgia, dyspnea, pleuritic chest pain, diarrhea, and delirium. The white blood cell count is normal or elevated, with an increased proportion of immature neutrophils. Chest radiographs of patients with Legionnaires' disease typically show alveolar infiltrates, although a variety of radiographic patterns may be seen. Pleural effusions, when present, are typically small. Cavitation of pulmonary infiltrates occasionally occurs, particularly in immunosuppressed patients.

The severity of illness in legionellosis ranges from asymptomatic seroconversion to rapidly fatal pneumonia. Among patients with at least a 4-fold rise in antibodies to *Legionella* after admission to hospitals where ongoing nosocomial transmission was occurring, 29–69% had no evidence of pneumonia.[56] Mortality in Legionnaires' disease outbreaks has ranged from 5 to 30%.[91] Among cases reported through surveillance, 24% were fatal.[132] Death occurs due to progressive pneumonia with hypoxemia and shock. Factors associated with a fatal outcome include advanced age, immunosuppression, nosocomial acquisition, end-stage renal disease, and cancer.[132] Historically, the drug of choice for treatment of Legionnaires' disease was erythromycin. However, reports of treatment failures with erythromycin have led to the evaluation of other antibiotics[163] and a change in the treatment recommendations. Infectious Diseases Society of America guidelines for treatment of community-acquired pneumonias recommend the use of fluoroquinolones or azithromycin for patients with Legionnaires' disease.[126] Convalescence following Legionnaires' disease may be prolonged in some cases, with weakness and shortness of breath persisting for months. Residual defects in pulmonary diffusing capacity have been observed.[116] Reinfection with *L. pneumophila* has been reported for severely immunocompromised patients.[118] Manifestations of disease caused by *L. pneumophila* and by other species of *Legionella* appear to be similar.[64]

Extrapulmonary infections during the course of Legionnaires' disease have been recognized, most likely due to bacteremic seeding during the course of pneumonia. Reported extrapulmonary infections with *Legionella* spp. in the absence of clinically apparent pneumonia include involvement of the endocardium, pericardium, peritoneum, bowel, respiratory sinuses, and skin and soft tissues.[60,61] Extrapulmonary disease generally occurs in immunosuppressed patients.

Pontiac fever is an acute illness characterized by fever, headache, and myalgia. Cough, sore throat, and chest tightness are observed in about 50% of cases.[79] Other symptoms occasionally reported include dizziness, nausea, confusion, and a burning sensation in the eyes. No specific therapy is required for Pontiac fever. Illness may be debilitating for several days, but recovery is usually complete within 1 week. Prolonged effects of Pontiac fever have been suggested though studies with sufficient follow-up are limited.[36]

8. Control and Prevention

8.1. General Concepts

The risk of developing *Legionella* infection from an aquatic environment depends on several factors including conditions that support amplification of the organism, a mechanism of exposure (e.g., aerosolization of colonized water), and transmission to hosts with risk factors for development of disease. Approaches to the control and prevention of legionellosis involve strategies that may interfere in any of the steps from colonization of a water system by *Legionella* to transmission of disease.

The approach to the prevention of legionellosis can take two forms. Primary prevention involves the control of *Legionella* in a water system before the detection of disease. Secondary prevention focuses on eradication of *Legionella* from aquatic systems once cases of legionellosis have been recognized.

Strategies for primary prevention should create conditions that are unsuitable for bacteria amplification and dissemination to occur. Certain characteristics of legionellae can be exploited to control and eradicate the organism. Legionellae grow poorly at temperatures below 20°C or above 50°C and are readily killed at temperatures above 60°C.[67] Additionally, legionellae are susceptible to a broad range of disinfectants, including chlorine- and bromine-containing compounds, ozone, heavy metal ions, and ultraviolet light.[115,143,172] Sound engineering design and regular maintenance of water systems are also key to the control of *Legionella* growth. The American Society of Heating, Refrigeration, and Air-Conditioning Engineers (ASHRAE) has issued guidelines for the control of *Legionella* in buildings. For cooling towers ASHRAE recommends regular maintenance, the use of a biocidal treatment program, and the use of the services of a qualified water treatment specialist to determine and oversee the treatment program (8). Additional strategies for primary prevention include placement of towers as far from building air intakes and public areas as possible and the use of drift eliminators to minimize aerosol dissemination.[31]

For secondary prevention in situations where cooling towers have been implicated in outbreaks of Legionnaires' disease, remediation can be complex and requires the assistance of experts in these processes. Methods that have been successful in preventing further transmission in outbreak settings include hyperchloridation and mechanical cleaning.[180]

A number of methods have been used to eradicate legionellae from potable water systems.[144,180] In buildings where the heated water system has been identified as the source of Legionnaires' disease outbreaks, those systems have been decontaminated by thermal disinfection (i.e., flushing each distal outlet of the hot water system with water at 71–77°C) or hyperchlorination (flushing all outlets of the hot water system with water containing 1–2 mg/l free residual chlorine at the tap), and transmission has been interrupted. Following either of these procedures, hot water should be stored at a temperature 60°C and circulated with a minimum return temperature of $\geq$51°C. Cold water should be stored and circulated at $<$20°C.[7] Disadvantages of thermal eradication include the risk of scalding. Supplemental chlorination has caused accelerated corrosion and leaks in plumbing systems and could lead to formation of potentially carcinogenic halogenated organic compounds.[144] Additional measures, such as physical cleaning or replacement of hot water storage tanks, water heaters, faucets, and shower heads, may be required to remove accumulated scale and sediment that protect organisms from the biocidal effects of heat and chlorine. Plumbing system design may need to be altered to remove portions not reached by thermal eradication and chlorination and where amplification may continue to occur (e.g., spur pipes).[151] Experience with alternative methods for control and eradication of legionellae in water systems, such as treatment of water with ozone, ultraviolet light, or heavy metal ions, has shown some promising results but is more limited. There is, however, an increasing volume of epidemiologic and laboratory data that suggest that the use of monochloramine as a biocide in municipal water systems is more effective than chlorine in preventing *Legionella* transmission.

Monochloramine is a residual disinfectant that is more stable than chlorine allowing maintenance of active concentrations in larger areas of water distribution systems. Furthermore, it results in reduced production of regulated disinfection by-products such as total trihalomethanes (TTHMs). In 1998, the US Environmental Protection Agency (EPA) issued regulations requiring the reduction in the levels of certain disinfection by-products including TTHMs. Since then, many municipalities are converting to monochloramine as the primary residual disinfectant for their public water distribution systems. In 2004, a survey of municipal water companies in the United States showed that 30% used monochloramine as their residual disinfectant.[168] There are several studies that show a decrease in transmission of *Legionella* when monochloramine is used. In 1999, a case–control study assessed the risk of a hospital having a potable water-associated outbreak of Legionnaires' disease, based on the type of disinfectant used by the municipality.[113] Hospitals that experienced an outbreak were 10 times more likely to be in municipalities that used chlorine as the drinking water disinfectant compared to municipalities that use monochloramine as the disinfectant. These results suggested that approximately 90% of the outbreaks associated with drinking water could have been prevented by a change at the water treatment plant. This study as well as others showed promise for the potential impact of monochloramine in the prevention of *Legionella*. As municipal water companies have switched to monochloramine, there has also been the opportunity to look at the impact of monochloramine prospectively. In Pinellas County, Florida, 96 buildings were tested for *Legionella* before and after the conversion to monochloramine. After the conversion, the percentage of buildings colonized with *Legionella* declined from 19.8 to 6.2%.[140] Results from a similar but larger survey in San Francisco supported this reduction in *Legionella* colonization with monochloramine use.[69] Monochloramine holds promise for the prevention of Legionnaires' disease caused by potable water. Further prospective studies that examine not only colonization but reduction in disease, such as in hospital settings, are needed.

An alternative approach to control is based on periodic, routine culturing of water samples from cooling towers, potable water systems, or other aerosol-producing devices and instituting control measures if legionellae are detected.[4,195] However, this method has not been widely recommended by public health officials because (1) the results of water culture do not necessarily correlate with the potential for transmission; (2) the bacterium is frequently present in water systems without being associated with known cases of disease; (3) interpretation of the results of routine culturing of water is confounded by use of different bacteriologic methods in various laboratories, by variable culture results among sites sampled within a water system, and by fluctuations in the concentration of legionellae isolated from a single site[16,128,130]; and (4) the risk of illness following exposure to a given source is influenced by other factors (e.g., strain virulence, host susceptibility).

The Centers for Disease Control and Prevention (CDC) has published guidelines regarding environmental infection control in health care facilities.[167] They provide no recommendation regarding routine water sampling in hospitals, due to the concerns outlined above, except in the setting of transplants units, where routine water sampling is considered reasonable if it is conducted as part of a comprehensive Legionnaires' disease prevention strategy. In health care settings without transplant units, CDC promotes an approach with primary emphasis on patient surveillance. If one laboratory-confirmed case of hospital-associated Legionnaires' disease or two possible nosocomial, laboratory-confirmed cases occur within a 6-month period, additional measures should be taken including increased retrospective and prospective surveillance for possible cases. The extent to which environmental sampling and remediation occur is dependent on whether there is ongoing transmission of *Legionella* and the links between the epidemiologic and environmental findings.[167] Comparison of isolates from patients and environmental samples, along with appropriate epidemiologic data, allows determination of the source of the disease and appropriate remediation to prevent further cases. Confirmed cases of Legionnaire's disease should be reported to local and state public health authorities.

8.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis

Erythromycin chemoprophylaxis of immunosuppressed patients at a hospital with a high incidence of nosocomial Legionnaires' disease due to colonization of the hot water distribution system resulted in protection against Legionnaires' disease.[187] However, in view of the generally low attack rate during outbreaks and the potential of eliminating the source of the organism, chemoprophylaxis is seldom indicated except perhaps in settings such as transplant units. An alternative measure in hospitalized patients is to empirically treat with fluoroquinolones immunocompromised patients who develop pneumonia of uncertain etiology.[171]

8.3. Immunization

Although animal models with vaccines for *Legionella* have shown some success, the development of vaccines for humans against legionellosis has not been attempted.

9. Unresolved Issues

9.1. Rapid Diagnostic Tests

The clinical presentation of Legionnaires' disease is similar to that of other types of pneumonia; therefore, accurate, rapid diagnostic tests are essential to ensure appropriate clinical management of patients and early recognition of outbreaks so that prevention strategies may be instituted. Continued development and evaluation of new diagnostic modalities, such as antigen detection tests and polymerase chain reaction, are needed. Tests to detect *Legionella* other than *L. pneumophila* serogroup 1 antigens in urine may become available in the near future.[181] Additional studies are needed to determine whether polymerase chain reaction is as sensitive and specific as culture for identifying *Legionella* in clinical specimens and whether the effect of substances present in sputum that inhibit the assay can be overcome.[60,61]

9.2. Risk Assessment

Although environments capable of supporting the growth of *Legionella*, such as cooling towers and hot water distribution systems, are a daily part of life and the bacterium is frequently present in these systems, transmission resulting in disease occurs relatively infrequently. More information is needed about the specific virulence, host, and environmental factors that increase the risk of transmission. Epidemiological studies in concert with careful environmental investigations can define the risk of Legionnaires' disease attributable to various colonized water systems and aerosol-producing devices. The role of environmental monitoring in risk assessment is yet to be determined, and at present, limited data are available on the association between the concentration of organisms in a water system and the risk of transmission.[16,170] The utility of nonculture methods for

detecting virulent *Legionella* in the environment needs to be further defined.[3,112,124,189] Additionally, the pathogenic potential of *Legionella* and related organisms that are not detected by culture methods designed to detect *Legionella* needs to be assessed.[85,138,159]

9.3. Prevention

Prevention of legionellosis is challenging because of the steps necessary to identify an individual source of transmission, because of the diversity of settings in which the disease occurs, and because of the difficulty in eradicating *Legionella* from aquatic environments. To identify the source of transmission to an individual case, clinicians must diagnose Legionnaires' disease in an individual who presents with community-acquired pneumonia, the case must be reported to public health authorities, and public health authorities must be able to identify the most likely source among the many that are determined to be colonized with *Legionella*. For community-acquired cases, including those associated with travel, the number of potential sources to which an individual is exposed is usually too large to allow definition of a clear epidemiologic link between a specific source and the individual case. Even when the number of potential sites is limited, such as in a hospital, identifying the source of transmission can be challenging because of the lack of sufficient epidemiologic or laboratory data to implicate that source. Finally, remediation of water systems is rarely straightforward and recolonization is common.

10. References

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CHAPTER 20

Leprosy

Richard I. Frankel and David M. Scollard

1. Introduction

Leprosy (Hansen's disease [HD]) is a chronic infectious disease caused by *Mycobacterium leprae* that primarily affects the skin and peripheral nerves. A number of other organs, including eyes and testicles, may be involved in the infection. HD has been known for over 2000 years and has afflicted millions of people worldwide. In recent times, most patients have been residents of underdeveloped nations. Hansen[1] discovered the etiologic agent over 130 years ago, but it is still not possible to culture this bacterium on solid medium or to propagate it in liquid medium. This has hampered investigation into the pathogenesis of leprosy and there is much about the disease process that we do not understand.

As discussed in Section 8, leprosy may have a variety of clinical presentations, depending on the patient's immune response to the causative organism. The initial clinical form is referred to as indeterminate. The indeterminate lesion is usually single and often heals spontaneously. If the lesion does not resolve spontaneously and the patient is not treated, the disease usually remains localized in patients with a high degree of immunity to *M. leprae* and evolves into tuberculoid disease. If the immune response to *M. leprae* is poor, more extensive (borderline or lepromatous) disease will result. Patients with intermediate degrees of immune response will have intermediate clinical presentations. The terms TT, BT, BB, BL, and LL are commonly used to indicate the polar tuberculoid, borderline tuberculoid, mid-borderline, borderline lepromatous, and polar lepromatous forms of leprosy, respectively. These are described in Section 8. It is also important to be familiar with the WHO classification of patients into paucibacillary (PB) or multibacillary (MB) leprosy. The former is defined as a person with no bacilli detected on slit skin smears (if such an examination is done) or with five or fewer skin lesions. The latter is defined as a person with bacilli detected on slit skin smears or with more than five skin lesions.

While most infectious diseases are characterized by a relatively well-defined incubation period, this is not the case with Hansen's disease. The major reasons for this are lack of historical information to determine the time of infection, lack of a clinical test to determine the time of infection, and difficulty in determining the time of onset of clinical disease. In addition, it is very likely that many, if not most, patients experience a period of latent infection, with disease only appearing when some internal or external factor affects the individual's ability to resist *M. leprae*. This is analogous to human infection with *M. tuberculosis* and probably accounts for those patients reported to have extremely long incubation periods. Rare cases in infants may indicate that the incubation period can be as short as a few weeks, though it is impossible to rule out intrauterine infection in these cases.[2] Most studies suggest that the typical incubation period is from 2 to 10 years, and it is likely that most who do not experience latent infection have an incubation period from 2 to 5 years.[3,4]

As with any uncommon disease, clinicians in the United States often have a low index of suspicion for leprosy and the diagnosis is often made relatively late. The variable immune response and the resulting variable clinical manifestations often present challenges in diagnosis and treatment. An understanding of the clinical manifestations of the infection itself, the clinical manifestations of the immunologic components of the disease, and the problems associated with treatment are essential for proper care of patients. While the factors mentioned above have also hindered progress in control of the disease, nevertheless, great progress has been

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made in the last 20–25 years. Much of this improvement has been due to the adoption of multidrug therapy of fixed duration and the World Health Organization (WHO) campaign to eliminate leprosy as a public health problem. Nevertheless, leprosy continues to be a problem in a number of countries and many patients who are no longer counted as cases of leprosy still suffer and require care to manage the sequelae of their infection. In addition, while much progress has been made, some patients continue to suffer from the stigma associated with leprosy. Hence many people prefer to use an alternate term such as Hansen's disease or hanseniasis.

2. Historical Background

It is uncertain just where and when leprosy first appeared and whether biblical "leprosy" was in fact leprosy as we know it today. However, the opinion of some authorities[5] has been that it started in the Indian subcontinent before 600 BC and then spread initially to Asia and Africa and later to Europe. Geographic spread has been associated with the movement of people associated with the spread of religion and the movement of people associated with military campaigns. A recent study, utilizing genetic markers for strains of *M. leprae*, suggests that the disease originated in East Africa or the Middle East (the authors use the term "Near East") and spread from there to Europe and North Africa.[6] The incidence of the disease in Europe probably peaked in the Middle Ages and then gradually declined, virtually disappearing in most of Europe, though it is still found in some countries that border the Mediterranean Sea. The reasons for this disappearance are uncertain, but it is very likely that leprosy, like tuberculosis, is significantly impacted by socioeconomic conditions and that improvement in hygiene, housing, and nutrition played a major role in the waning of the epidemic.[5]

Leprosy was probably introduced into the Americas by the Spanish and subsequently other colonialists and also by means of the slave trade. The first definite reference to the disease in the United States was in Florida[7] in 1758. In the 1760s, cases occurred among the French near the mouth of the Mississippi River. In 1785, a hospital was established in New Orleans, Louisiana, for the treatment of leprosy patients, and over 100 years later (1894), this facility was moved to a location near the village of Carville, becoming the Louisiana Leper Home. Patients from many states began seeking care there, leading the federal government to acquire the institution in 1921. It was initially called the National Leprosarium, later the Gillis W. Long Hansen's Disease Center (GWLHDC) and subsequently the National Hansen's Disease Program (NHDP). The residential treatment program of the center ended following federal legislation passed in 1998.[8] In addition, outpatient treatment, rehabilitation, training, and research moved to Baton Rouge, Louisiana. Each year, a few US-born individuals are diagnosed with Hansen's disease. Most patients in the United States acquire their infection in other countries and transmission within the United States is rare. Cases continue to occur in immigrants from countries where the disease is still prevalent.[9] Hawai'i, California, Texas, Louisiana, Florida, and the New York City area report the most cases. In the 19th century, there were many cases in Scandinavian immigrants residing in and around Minnesota, but this focus has now completely disappeared. A settlement for patients in Hawai'i was the result of quarantine on the island of Moloka'i, which began in the 19th century. No new patients have been sent to the settlement, now called Kalaupapa, since 1969. Not all of the patients eligible to reside there do so, and some reside there on a part-time basis. There were 25 patients eligible to live there as of October 13, 2008, with the number steadily decreasing due to deaths in a population that is now elderly. Another US leprosy settlement on Penikese Island, Massachusetts, closed in 1921.

Significant milestones in the history of leprosy include the discovery by Hansen[1] of *M. leprae* as the causative agent of this disease in 1873, the introduction of sulfone therapy in 1941,[10] the development of the mouse footpad technique for growing the bacillus in 1960,[11] recognition of the immunologic basis of the spectrum of pathologic changes in leprosy in 1964,[12] the discovery that the bacillus grows readily in the armadillo in 1971,[13] the development of fixed duration antimicrobial therapy in 1981,[14] and the cloning of the *M. leprae* genome in 2001.[15]

3. Methodology

3.1. Sources of Data

3.1.1. Sources of Mortality Data. Leprosy is rarely a fatal disease and thus mortality data are not useful in understanding the disease. Fatalities related to leprosy almost always result from secondary bacterial infection of wounds related to dysfunction of sensory and, to a lesser extent, motor nerves. Rarely, individuals may die from renal dysfunction related to a type 2 Hansen's disease immunologic reaction (Gell–Coombs type 4) or to renal amyloidosis or from an adverse reaction to medication. Prior to the availability of effective antimicrobial therapy, laryngeal involvement was fatal for some patients.

3.1.2. Sources of Morbidity Data. Although many countries list leprosy as a reportable disease, existing

morbidity data on leprosy are often unreliable. There are still varying degrees of stigma attached to the disease in most countries, so that some patients may try to either conceal the diagnosis or seek the care of physicians who will not report it. In addition, the disease has traditionally been difficult to diagnose and cases are often missed. As the disease has become less common, fewer health care workers are familiar with it and thus the diagnosis may be missed more often. The World Health Organization (WHO) collects data and publishes annual summaries and periodic updates.[16] WHO reports registered cases at the beginning of the year and the number of cases detected during the preceding year. There are two important caveats. First, WHO defines a registered case as a person with leprosy who has not completed a full course of treatment. Patients who have residual disability or who have immunologic complications but are not felt to require antimicrobial therapy are not included in WHO prevalence data. Thus, these data underestimate both the burden of suffering and the burden to the health care system. Second, although case detection rate is used to approximate the incidence rate, the factors listed above result in an underestimation of the true incidence rate in many countries.

In the United States, leprosy is a reportable disease in most states and it seems likely that almost all cases are reported to the national registry at the National Hansen's Disease Program (NHDP) and to the Centers for Disease Control and Prevention (CDC). Cases are reported on a weekly basis by CDC in the *Morbidity and Mortality Weekly Report* under "Provisional cases of infrequently reported notifiable diseases (<1,000 cases reported during the preceding year) – United States"[17] and annually by NHDP electronically.[18]

3.2. Surveys

Surveys to detect new cases of leprosy and to assess prevalence have primarily been done by clinical examination of defined populations. The most common of these have been household contact examinations, but community surveys, school surveys, and examination of special groups have been productive in certain circumstances.[19] In actual practice, most cases are found by voluntary reporting or referral by medical personnel. In highly developed countries where the disease is rare, examination of household and other very close contacts is the only kind of survey that has any practical value.

There has been considerable confusion about the lepromin skin test. Although it is no longer useful clinically, we include this discussion because there are frequent references to it in the literature, primarily the older literature. Some have erroneously viewed it as analogous to the tuberculin skin test for tuberculosis. In fact, the lepromin skin test is not useful as a screening test for leprosy. The test measures the person's cell-mediated immune response to killed *M. leprae*. It has a very low sensitivity in leprosy patients at the lepromatous end of the spectrum, in whom *M. leprae*-specific cell-mediated immunity (CMI) is uniformly low or absent. It has a very low specificity in leprosy patients at the tuberculoid end of the spectrum, as there is cross-reaction to infection with other mycobacteria. Thus, the lepromin test is not useful for detecting leprosy cases in populations or in individuals. It is also no longer necessary or useful for clinical classification of leprosy. It is presently only used in research studies. For historical purposes, it is worth noting that lepromin was initially prepared from tissue of human patients with leprosy. Since the development of the armadillo model, virtually all lepromin is now prepared from armadillo tissue and is known as lepromin A. A purified preparation known as Dharmendra lepromin has been used in India. For lepromin testing, a single 0.1-ml dose of the preparation was injected intracutaneously, and readings were taken at 48 hours (Fernandez reaction) and at 3–4 weeks (Mitsuda reaction). There was some correlation of the Mitsuda reaction with clinical findings but, as noted, the test is no longer used. Other tests of the immunologic response to *M. leprae*, including tests of lymphocyte response to *M. leprae* antigens and antibody assays, have been used in surveys. The tests of cell-mediated immune responsiveness have the same limitations as the lepromin test and are not useful in the management of patients.

Of the various antibody tests that have been developed, the one that has been most widely studied is detection of antibody produced in response to phenolic glycolipid-I (PGL-I). This glycolipid is found in the capsule of *M. leprae*. It contains a trisaccharide moiety that has not been found in any other bacterium. Thus, antibodies to PGL-I should be specific for infection with *M. leprae*. Antibodies to PGL-I are usually present in patients with generalized forms of leprosy, i.e., BL and LL disease. Diagnosis in this group of patients can be readily made by standard clinical tests, however. Antibodies are sometimes found in patients with localized forms of leprosy, i.e., TT and BT disease, but the sensitivity of the test is low if used for diagnostic purposes. For example, one study found that antibodies were present in 93.1% of patients with a bacterial index of 4.0–5.0 but in no patients with bacterial index of 0–1.9.[20] Likewise antibodies are found more often in contacts of patients with leprosy than in the general population, but there is limited correlation between finding antibodies in an asymptomatic individual and the likelihood of that individual subsequently developing clinical leprosy.[21] Thus, detection of anti-PGL-I antibodies is not used for clinical diagnosis but only in research studies.

The most recent development in this area is the use of the polymerase chain reaction (PCR) as a direct test for

the presence of *M. leprae* DNA. A study[22] of nasal swabs taken from patients in the Philippines showed that the swabs were PCR positive for *M. leprae* in 55% of untreated leprosy patients, in 19% of persons who worked with leprosy patients daily, and in 12% of persons who worked in the same area but had no direct contact with leprosy patients. In a study in Indonesia, *M. leprae* was detected by PCR in 7.8% of nasal swabs among all subjects in a population-based study of two villages with a high prevalence of leprosy, with no correlation between the PCR results and serologic testing for IgM antibodies to PGL-I.[23] While these studies have been valuable in demonstrating frequent nasal carriage of *M. leprae* in asymptomatic individuals, they have not been useful in terms of screening for disease and are not likely to be used in this manner in the future.

3.3. Laboratory Diagnosis

In developed countries, the only diagnostic test for leprosy is a skin biopsy. This is generally a punch biopsy. There are three caveats with regard to obtaining and processing the biopsy. First, for annular and plaque-type lesions, the biopsy must be obtained from the margin of the lesion rather than the center. The central clearing that is observed clinically may be accompanied by loss of the typical histopathologic features. Second, the biopsy should be full thickness, down to the subcutaneous fat, as sometimes the diagnostic findings are localized to the deep dermis. Finally, the Ziehl–Neelsen stain should not be used when looking for *M. leprae*. This bacterium is less acid-fast than *M. tuberculosis* and thus may not be seen with a Ziehl–Neelsen stain. Instead, a modification of the Ziehl–Neelsen stain is necessary. The Fite–Faraco method, which enhances the weak acid-fastness of *M. leprae*, is most often used. The stain is commonly called the Fite stain.

A major recent addition to laboratory diagnostic capabilities for leprosy is the development of the polymerase chain reaction (PCR) assay for *M. leprae* DNA.[24] This test can be performed on formalin-fixed, paraffin-embedded tissue as long as exposure to formalin is not greater than 24 hours. The test is frequently negative when few bacilli are present. It is therefore used to determine which mycobacterium is present when organisms have been identified in Fite–Faraco-stained sections and it does not replace the standard microscopic examination of Fite–Faraco-stained sections.[25]

3.3.1. Isolation and Identification of Organism. Unlike most pathogenic microorganisms, there is no diagnostic culture method for *M. leprae*, although PCR identification of *M. leprae* DNA is possible, as noted above. In the past, slit skin smears were important in diagnosis and contributed information that was helpful in making therapeutic decisions. Skin smears involved obtaining fluid and some tissue from the dermis and examining it for acid-fast bacilli. In highly developed countries with a low incidence of leprosy, skin biopsies are virtually always done for diagnosis. Skin smears are therefore seldom useful in these places. While still of some use in situations where skin biopsies are not routinely done, the World Health Organization no longer recommends them for routine use but relies instead on its clinical classification system. Aside from the diminished clinical value of the smears, there has been a problem with reproducibility of results, which has also reduced their clinical utility.

For historical purposes and understanding of older literature, it is useful to know that slit skin smear results were quantified by reporting the number of bacilli seen using a semilogarithmic scale called the bacterial index or BI. The results ranged from zero to six. In addition, the percentage of acid-fast bacilli that were intact was reported as the morphologic index or MI. Of particular interest is the fact that the MI was usually 1–5% and virtually never over 20%, even in the untreated patient with diffuse lepromatous leprosy.

As initially reported by Shepard in 1960,[11] *M. leprae* will grow in the mouse footpad. This discovery was a monumental breakthrough in terms of leprosy research and in assessing and demonstrating the efficacy of antimicrobial agents. It was one of the key scientific elements that led to the end of isolation of leprosy patients in the late 1960s. Growth in mouse footpads has not had and does not have a role in terms of diagnosis of leprosy.

Although *M. leprae* cannot be cultured in vitro, there are now several studies reporting limited metabolism in cell-free in vitro radiorespirometric systems. The method most studied is a system that measures $^{14}CO_2$ evolution from ^{14}C-labeled palmitic acid, such as the BACTEC and Buddemeyer systems, utilizing nude-mouse-derived *M. leprae*. These techniques have been used to screen potential antileprosy drugs and are a significant advance from using animal models.[26] Recent studies show that radiorespirometry may also be utilized to assess viability of *M. leprae* in biopsies taken from patients during treatment and could potentially be used to quantify response to antibacterial treatment.[27] Again, in vitro culture has no role in the diagnosis of leprosy.

3.3.2. Serological and Immunological Tests. Most aspects of serological and immunological tests have been reviewed in Section 3.2 above. For practical purposes, such tests are not useful in the diagnosis of leprosy. There have been numerous studies of PCR as a possible method of diagnosis. In general, most patients in whom AFB are demonstrated in routine biopsies also are positive for *M. leprae* on PCR testing. In these patients, PCR does not facilitate the

diagnosis. In those biopsies in which AFB are rare or negative, where only the presence of granulomatous infiltrate and involvement of peripheral nerves suggest the diagnosis of leprosy, only 50–60% are positive for *M. leprae* by PCR. When positive, PCR can consistently distinguish *M. leprae* from other mycobacterial species.[25,28] This is useful in those few situations where there may be diagnostic confusion between leprosy and infection due to so-called atypical mycobacteria. PCR for *M. leprae* is highly specific and sensitive and offers a new approach to identifying *M. leprae* under clinical conditions.[29] It seems unlikely, however, that this test will play a significant role in most countries.

4. Biological Characteristics of the Organism

Although *M. leprae* organisms cannot regularly be isolated and positively identified from all patients with leprosy but only from patients with high bacterial counts, and the Henle–Koch postulates have therefore not been and likely will not be fulfilled, almost all believe that *M. leprae* is the causative agent of leprosy. *M. leprae* was first described in 1873 and was the first bacterium to be identified as a cause of human disease. However, with the exception of the limited growth mentioned above, it has not been propagated in any artificial medium and has never been grown on solid medium. Thus the usual methods for studying bacteria have not been applicable. Consequently, less is known about this bacterium than most other bacteria of medical importance.

M. leprae is a gram-positive acid-fast bacillus, 0.3–0.4μm × 2–7μm. It is an obligate intracellular parasite. It is the only bacterial species with a special predilection for peripheral nerves and specifically for Schwann cells. On skin smears or biopsy sections, the bacilli are often clustered in oval formations called globi. Over 90% of the bacilli from a typical new patient stain irregularly and appear to be nonviable in mouse footpad studies.[30] With effective chemotherapy, all bacilli become granular and disintegrate. Although the disintegrating bacilli appear to slowly clear with treatment, this clearing may be due in part to a loss of acid-fastness by the bacillary remnants rather than to actual disappearance of the bacilli. Staining with the Gomori methenamine silver technique has demonstrated bacilli in some biopsy sections that had shown none on a Fite–Faraco stain.[31]

M. leprae will survive in soil for up to 33 days[32] and in dried secretions for up to 9 days in a hot, humid climate.[33] At the temperature of wet ice, they may survive 2 weeks or more, while heating at 45°C for 1 hour[34] or freezing[35] kills them. In experimental systems in vitro, these bacilli die much more rapidly at 37°C than at 33°C. The antigenic activity of cell wall antigens persists at temperatures of over 200°C.[36] In the mouse footpad, maximum growth occurs at an average footpad temperature in the 27–30°C range.[37] This corresponds to its growth pattern in humans where the highest bacterial counts occur in the cooler areas of the body such as the skin, extremities, and the anterior portion of the eye. In the mouse footpad, there appear to be "fast"- and "slow"-growing strains, but it is not clear if these characteristics have any bearing on human disease. A number of specific *M. leprae* antigens have been identified using monoclonal antibodies. The most thoroughly investigated from a clinical point of view is PGL-I, discussed above.[21] There are also many non-specific *M. leprae* antigens.

The large number of relatively tissue-free bacilli obtainable from armadillos has made it possible to prepare *M. leprae* DNA libraries in *Escherichia coli* vectors and to sequence the entire genome,[15] enabling application of some of the techniques of molecular biology to the study of this organism. When its genome is compared to its close relative, *M. tuberculosis*, it is apparent that *M. leprae* has undergone an extreme case of reductive evolution.[38] This is reflected by the smaller genome (3.3 Mb for *M. leprae* versus 4.4 Mb for *M. tuberculosis*) and a major reduction in G+C content (58% for *M. leprae* versus 66% for *M. tuberculosis*). *M. leprae's* annotated genome contains only 1,614 open reading frames (ORF) encoding potentially functional proteins, compared to 3,993 ORF in *M. tuberculosis*. In addition, the *M. leprae* genome contains 1,133 "pseudogenes" (inactivated genes whose function has been lost through mutation), compared to only 6 pseudogenes in *M. tuberculosis*.[39] Complementing the work on sequencing the genome of *M. leprae* is work on sequencing the genome of *Dasypus novemcinctus*, the nine-banded armadillo. This will almost certainly yield important information regarding the unique susceptibility of this animal to lepromatous leprosy. Adams and coworkers recently reported success in expressing armadillo interleukin-2 in *E. coli*.[40]

To identify genes transcribed during infection, gene transcripts from *M. leprae* growing in athymic nude mice have been studied using an *M. tuberculosis* microarray.[41] The transcripts detected thus far indicate that *M. leprae* actively catabolizes fatty acids for energy. In addition, the bacillus produces a wide range of secretory proteins, utilizes the limited array of sigma factors available (a sigma factor is a protein component of RNA polymerase that determines the specific site on DNA where transcription begins), produces several proteins involved in the transport, storage, and regulation of iron, and transcribes several genes associated with virulence in *M. tuberculosis*.[42]

The proteome of *M. leprae* has been evaluated using subcellular fractionation of *M. leprae*, combined with

immunologic screening (using serum and T-cells from leprosy patients and contacts) and examination of purified proteins by micro-sequencing and mass spectrometry. Proteins have been identified from major cellular compartments including cytosolic, membrane, and cell wall. The results have been summarized.[43] Analysis indicates that *M. leprae* has lost many metabolic pathways together with their regulatory circuits, particularly those pathways with catabolic potential.[38] Secreted proteins of *M. leprae*, previously not accessible in this non-cultivable organism, can now also be studied using molecular techniques and bioinformatics analysis. The results thus far indicate that *M. leprae* does have the potential to produce a variety of secreted proteins,[41] but most of these have yet to be studied regarding their role in pathogenesis, value for diagnostic testing, or potential use as immunostimulating agents for therapeutic or preventive use.

5. Descriptive Epidemiology

As noted in Section 3.2, most of the current epidemiologic data come from the World Health Organization. US data come from the Centers for Disease Control and Prevention and the National Hansen's Disease Program.

5.1. Prevalence and Incidence

There have been dramatic changes in the global number and distribution of cases of Hansen's disease in the past two decades. The terms "incidence" and "prevalence", as applied to leprosy, refer to the number of new cases registered and the total number of cases registered, respectively. As a result, they are subject not only to changes in the occurrence of the disease in the population studied but also to marked change as a result of operational factors affecting the specific registry. In considering these data, then, the reader must be aware of certain factors. First, incidence often varies widely within a given country and sometimes even within smaller regions such as provinces, states, or smaller sectors. In addition, leprosy, as with many diseases and especially with uncommon and/or potentially stigmatizing diseases, is underdiagnosed and underreported. Thus, case detection rate is reported rather than incidence rate. Third, WHO data include only those persons diagnosed with leprosy who have not completed a course of antimicrobial therapy. They do not include patients who have completed antimicrobial therapy but have residual physical and/or emotional disability. This group of patients may require significant health care resources for treatment, education, rehabilitation, and psychosocial support.

Global leprosy prevalence had been increasing progressively through the 1970s and early 1980s. The number of registered cases was approximately 2.83 million in 1966 with an estimated prevalence of 10.8 million. There were 3.6 million registered cases in 1976 with an estimated prevalence of 10.6 million, and 5.37 million registered cases in 1985 with an estimated prevalence of 10–12 million. While some of this increase was likely due to increased case finding activities, there was clearly a true increase in cases. This trend, along with concern about increasing resistance of *M. leprae* to dapsone, led a WHO study group convened in 1981 to recommend in 1982[14] that all patients be treated with defined-duration multidrug therapy. The subsequent implementation of this recommendation has led to dramatic changes in prevalence. The number of registered cases fell to 1.67 million with an estimated prevalence of 2.43 million in 1994.[44] At the beginning of 1999, there were 731,000 registered cases.[45] At the beginning of 2004, there were 458,000 registered cases.[46] This number had fallen to 225,000 at the beginning of 2007.[16]

In 1990, the World Health Assembly recommended an international effort to eliminate leprosy as a public health problem by the year 2000.[47] The assembly defined elimination as a prevalence rate below 1 case per 10,000 population in every country and in the major subunits of highly endemic countries. By the beginning of 2005, all but nine countries had achieved the elimination target.[16] (There were 122 such countries in 1985.) These nine were Angola, Brazil, Central African Republic, Democratic Republic of the Congo, India, Madagascar, Mozambique, Nepal, and the United Republic of Tanzania. These nine countries, with a registered prevalence of 212,000 at the beginning of 2005, accounted for about 74% of all of the registered cases. India alone accounted for about 70% of the cases among the nine countries or 52% of the global total of registered cases. By the beginning of 2007, only Brazil, the Democratic Republic of the Congo, Mozambique, and Nepal had not yet achieved the elimination goal.[48] As the number of countries failing to meet the elimination target has diminished, WHO has moved from a strategy of targeting every country to one utilizing a Special Action Project for the Elimination of Leprosy (SAPEL) in each targeted country or specified geographic region.[49] Started in 1995, these programs focus on areas with high leprosy caseloads and/or inadequate leprosy services with the primary goal of improving delivery of multidrug therapy (MDT). Also in 1995, leprosy elimination campaigns (LECs) were established in selected countries, focusing on improving case detection.[49] New cases are, of course, then treated with MDT.[50]

While the prevalence was falling, there was concern about the failure of the case detection rate to decline. For

example, there were 584,412 new cases reported in 1991[51] and 653,354 new cases reported in 1992.[52] This number fell to 590,933 cases in 1993[44] but rose during the last half of the 1990s to a peak of 804,449 in 1998.[45] Initially it was assumed that the increase was due to increased case detection, better reporting, more patients seeking treatment, and other operational issues. Over time, however, more concern was directed toward such possible factors as the role of the person colonized with *M. leprae* but without clinical signs of leprosy and the role of non-human sources of infection. More recent data suggest that MDT and the associated programs established by WHO have in fact reduced the occurrence of new cases. The rate has fallen progressively to 514,718 cases in 2003,[46] 407,791 cases in 2004,[16] 299,036 cases in 2005, and 259,017 cases in 2007.[48] The importance of local epidemiology is emphasized by the fact that the decrease has been marked in the South-East Asian region of WHO and modest in the East Mediterranean and Western Pacific regions but has not been observed in the African or American regions. The fact that the South-East Asian region had almost 88% of the newly detected cases in 2001 and about 73% of the cases in 2004 demonstrates that improvement in this region will have a major global impact and that improvement in leprosy control is not evenly distributed.

The most recent US demographic data are for the calendar year 2004.[9] Notable findings include the registration of around 100–140 new cases each year since 1997. California, Texas, Hawai'i, and New York reported the most cases, 91 (70%) of the 131 total cases in 2004. About two-thirds of the cases were male, consistent with previous reports in this country. A total of 25 of the 131 cases (19%) were individuals born in the United States, the most of any country. The countries with the next greatest number of cases were Mexico (24), Micronesia <sic> (15), Brazil (12), and the Philippines (10). Only Texas (10), Louisiana (4), and Mississippi (2) had more than one US-born case. Nine states reported one US-born case each during that year. About one-third of the US-born cases were Hispanic, white ethnicity and one-third Asian or Pacific Islander, though the author of the report notes that some of the latter cases should have been reported in 2003. A total of 20 cases (15%) were white, not of Hispanic origin, but 16 of the 20 white, not of Hispanic origin cases were endemic, accounting for 64% of the endemic cases. It is difficult to get exact data for the United States, as registered cases are never removed from the national registry. Almost 12,000 cases had been registered as of October 2005. As of that date, there were 3,604 patients on the US registry who were currently receiving care, 2,146 of whom (60%) were receiving MDT. The US Centers for Disease Control and Prevention reports cases reported by states on an annual basis. The disease is not reportable in all states. The latest data for 2006 were published at the beginning of 2007.[17]

5.2. Epidemic Behavior and Contagiousness

Leprosy is often referred to as an infection with a low degree of contagiousness. In addition, statements such as "disease transmission takes place only after years of exposure" still appear in the literature. More recent data, primarily serologic data with support from PCR studies, demonstrate that *M. leprae* has a relatively high infectivity but a low virulence for most individuals.[53] Most infected individuals never have any evidence of disease. Were there a skin test comparable to the tuberculin skin test for diagnosis of latent infection, it is likely that the concept of infectiousness in leprosy would have evolved differently.

Epidemics in the usual sense of the word are rare with leprosy. While the spread of leprosy throughout Europe in the Middle Ages has been referred to as an epidemic,[5] many of the cases were probably not leprosy, the spread was gradual over a period of many years, and there is very little accurate information about it. The Nauru epidemic in the 1920s is the best-documented occurrence of a true leprosy epidemic.[54] Leprosy was reportedly introduced into this Central Pacific island by a woman who arrived there from the Gilbert Islands (now Kiribati) in 1911 or 1912. In 1920, the first case appeared among the native population, and by 1924, there were 284 cases in the population of about 1,200 individuals. Case finding was very active, with monthly examination of everyone on the island. By 1929, 438 cases had been found, and apparently the incidence gradually diminished thereafter. Thus, at the peak of the epidemic, about 35% of the population had been diagnosed as having leprosy. At the same time, many patients were noted to have cleared their infection and were then removed from the registry, so that 119 patients were discharged in 1928 alone. At the end of 1941, there were 133 patients on the island, 41 in the isolation facility and 92 being treated as outpatients. The Japanese controlled Nauru during much of World War II. They reportedly rounded up the remaining 41 patients who were confined in the isolation facility, sent them to sea in a ship and then sank the ship. Detailed data from 1950 show 12 cases in segregation and 56 treated as outpatients with a prevalence rate of 4.3%. Over 90% of the cases on Nauru were reportedly of the tuberculoid type, and deformities were uncommon. On the basis of the findings in Nauru, New Guinea, and elsewhere, Leiker[55] noted that when leprosy is introduced into a population which has not previously encountered *M. leprae*, what is essentially an "epidemic" of tuberculoid leprosy occurs, the disease spreads rapidly, there are no foci of infection,

i.e., particular villages and families with a higher incidence than others, and adults and children are equally susceptible. In old endemic areas, however, there is more lepromatous disease than in the newly infected population, a higher proportion of the cases are among children and young adults, the disease spreads slowly, and there are foci of infection. It is evident that the pattern of leprosy in Nauru (an extreme and unprecedented example) and in other newly infected populations does indeed fit Leiker's postulates. However, it is more difficult to characterize the situation in most countries on the basis of such a hypothesis, particularly since nearly all data are now reported to WHO in terms of PB and MB or unclassified disease. Nonetheless, WHO does report a rise in the proportion of MB cases in Africa, but PB leprosy has become more frequent in the Americas and Asia, and some districts in India report up to 70% of cases presenting with a single lesion.[44] The reasons for this are uncertain. In the most recent report from the United States,[9] 52% of cases were classified as lepromatous, 10% as borderline, and 33% as tuberculoid or indeterminate.

5.3. Geographic Distribution

Leprosy is often called a tropical disease. It is true that currently leprosy is virtually limited to tropical and subtropical regions. If one recalls that Hansen and others studied leprosy because it was a major health concern in Norway and one notes the spread of disease in Europe in the Middle Ages, it is clear that leprosy is not intrinsically a tropical disease, in the sense that trypanosomiasis spread by tsetse flies is tropical because the flies are found only in the tropics. As mentioned above, leprosy is distributed largely according to socioeconomic factors. Most of the underdeveloped countries where leprosy persists as a problem are in tropical or subtropical regions. Based on this, leprosy is found in these regions, but climate and latitude have nothing to do with this distribution.

5.4. Temporal Distribution

There is no significant variation in occurrence with the seasons. The association with tropical climates as a marker of countries with less developed economies has been mentioned above. The previously cited US report for 2004[9] noted a disproportionate number of cases reported in the first seven months of the year. Factors which may influence this pattern include contact tracing, increased vigilance for and awareness of Hansen's disease once a case has been diagnosed, clusters of cases in immigrants, and the vagaries of state reporting systems.

5.5. Age

While leprosy may occur in persons of virtually any age, there are some age-related factors that influence the incidence. Leprosy is uncommon in children below 2 years of age,[56] though occasional cases below the age of 6 months have been reported.[57] A significant number of cases below 5 years of age will be found in countries with a high or very high prevalence, indicating active transmission of the disease, and prevalence rates rise up to age 14, reaching values as high as and occasionally higher than those for the general population. Guinto[58] stated that "it is a fact that children are much more susceptible than adults", but Newell[59] believed that statistics in this regard could be equally well interpreted as indicating only that children have more opportunity for exposure. Findings in Goa and from some series in India and other countries demonstrate the great variations seen not only from country to country but also within the same country.[60,61] Generally speaking, the median age at onset is somewhere between 20 and 30 years. The most recent data from the United States[9] show that about 35% of the cases are in the age group of 25–39 years and 29% in the age group of 40–64 years at the time of diagnosis, with the oldest being 82 years. The mean age was 40.6 years and the median 37.5 years. The median age for the US-born cases was older at 56.8 years.

5.6. Sex

Sex ratios are generally 1:1 for children, but for adults the disease is usually more prevalent among males by a ratio of about 1.5–2:1, except in Africa where females predominate in some populations.[62] Cumulative US data through 2003 show a male:female ratio of 1.7:1.[63] The reason(s) for this predominance of adult males is unknown. Possible factors include genetic susceptibility and greater exposure outside of the household setting in most cultures. The US data, of course, are subject to possible gender differences in immigration.

5.7. Race

Although there are wide variations in incidence and prevalence and in the way the disease manifests itself throughout the world, there are no data that indicate any special race-related susceptibility or resistance. The previously mentioned variation in prevalence within countries and even within governmental jurisdictions within countries suggests that racial susceptibility is not a significant factor in the epidemiology of leprosy. Genetic considerations are discussed under Section 5.11 below.

5.8. Occupation

There is no particular relationship between occupation and the occurrence of leprosy. Some studies[64] have found an increased incidence of leprosy among missionaries working with leprosy patients; however, the prevalence was well below that in the population they were serving and was "increased" only over the prevalence in their homelands. In one study,[65] native hospital workers actually had less chance of contracting the disease than their counterparts in other occupations, and no cases have occurred among the staff at Carville. In this regard, the experience in Hawai'i is particularly interesting. Father Damien went to Moloka'i to care for the people with leprosy who had been exiled there. He arrived in 1873, the same year that Hansen first identified the bacillus that we now call *M. leprae*. Damien reportedly first noted a skin lesion in 1876 and apparently developed other symptoms in the 1880s. He was diagnosed with leprosy in 1885.[66] He died in 1889. He is the only health care worker in Hawai'i known to have contracted leprosy. One of us (RIF) has heard an anecdotal report of a second case but has never seen any confirmation of this.

5.9. Occurrence in Different Settings

The incidence of leprosy has generally been higher in family members where there is one or more cases in the family than in families with no cases, but considerable variation exists. Guinto[58] noted that, in a retrospective study done in the Philippines in 1935, the risk of contracting leprosy was eight times greater in family contacts of lepromatous patients than in the general population. In a follow-up prospective study (1935–1950), however, the risk was about five times, rather than eight times, greater. A study by Kluth[67] in Texas found that 2.6% of 1,522 household contacts of lepromatous patients developed leprosy. On the other hand, after a review of several other series, mostly foreign ones, Filice and Fraser[68] concluded that the long-term risk was such that about 10% of the household contacts of lepromatous patients would ultimately develop the disease. More recent studies have yielded similar data.[69] In all these studies, exposure to tuberculoid and probably indeterminate disease had an extremely small effect, i.e., the incidence of leprosy in families of tuberculoid and indeterminate cases was not significantly higher than in the general population. In US studies, the risks of exposed adults and children have been about the same, but in other countries, the risk for children is considerably higher.[68] The chance of a patient with leprosy infecting their spouse has also been evaluated[70] and seems to be about 5%. Institutional exposure can occasionally lead to very high rates. Of 2000 children born in or admitted to Culion Leprosarium in the Philippines in the 1930s without any sign of leprosy, 23% later developed the disease.[70] A large majority of these children experienced spontaneous healing of their lesions.[70] One must keep in mind that these studies all focus on contact with patients who had not been treated. As demonstrated in the seminal study by Worth[71] which was instrumental in ending quarantine for patients with leprosy, the risk in family members of a patient on effective therapy for leprosy is no greater than the risk of the general population in that community.

There was a report of 240 cases known to have occurred among US military veterans from 1940 through 1968.[72] The authors found that 46 of the cases were considered to have service-connected leprosy from exposure outside the United States during the course of their military duty. As the prevalence rate in the United States and globally has fallen since then, almost all new cases in US-born veterans, as in the rest of the population, would be expected to be acquired outside of the country.

The situation with regard to patients infected with the human immunodeficiency virus (HIV) is more complex. Since the initial case report,[73] there have been multiple case reports and a number of population-based studies[74,75] of the occurrence of leprosy in individuals with HIV infection. Overall, HIV infection does not seem to exert a significant influence on either the incidence or the clinical manifestations of leprosy, though not all studies are in agreement.[76] One possible exception to the lack of interaction between HIV and leprosy is the relatively recently observed immune reconstitution inflammatory syndrome in patients with leprosy and HIV who are treated with so-called highly active antiretroviral therapy (HAART) and respond to this treatment.[77,78] Clinically, this is probably a type 1 reaction, though the reaction may be more severe and more difficult to manage than the typical type 1 reaction not associated with HIV and its treatment.

5.10. Socioeconomic Factors

Leprosy today occurs mostly among the poor in underdeveloped countries. Why this is so is uncertain; however, crowded living conditions, poor diet, inadequate medical care, and improper sanitation may all play a role. As noted earlier, the incidence and prevalence of tuberculosis declined in many countries prior to the availability of antimicrobial chemotherapy. This was almost certainly due to better living conditions and better overall health. The same has occurred with leprosy and is consistent with the concept that the primary route of transmission of *M. leprae* is the respiratory route, as discussed in detail in Section 6. Good housing

conditions and extended schooling, for example, reduced the risk of leprosy in Malawi.[79]

5.11. Other Factors

Observations of familial clusters of leprosy cases, wide variation in prevalence and type of leprosy within a given population, high concordance rates of leprosy in identical twins,[80] and the persistence of anergy to *M. leprae* in patients with inactive lepromatous leprosy all suggested the existence of a genetic predisposition to leprosy.[81] Based on multiple studies in recent years, there is abundant evidence that genetics is critically important in determining the manifestations of leprosy. There is now a convincing body of evidence that indicates that different genes influence the human immune response to *M. leprae*, reviewed by Scollard et al.[42] These genes operate at two levels. The first level influences the overall susceptibility and resistance to this bacterium through its influence on "innate" resistance, mediated by cells of the monocyte lineage. If this innate resistance is insufficient to prevent infection and *M. leprae* becomes established in the individual, the second level of genetic control becomes important. This influences the degree of specific cellular immunity and delayed hypersensitivity generated by the infected individual. Such specific or acquired immunity is mediated primarily through the function of T lymphocytes, in cooperation with antigen-presenting cells. The association of genes with both innate and acquired immunity has recently been reviewed by Marquet and Schurr,[82] and genetic defects in different components of the type 1 cytokine pathway that affect human resistance to mycobacteria have been reviewed by van de Vosse et al.[83]

This recent demonstration of a marked genetic influence is not surprising, due to the accumulating evidence that genetic susceptibility is important in a number of infectious diseases.[84] This genetic influence likely impacts both whether or not the individual will develop any clinical manifestations of leprosy and whether the individual will develop disease toward the tuberculoid or the lepromatous end of the spectrum, although findings differ in various studies.[85,86,87] While mouse susceptibility to mycobacterial infection has been clearly linked to a specific protein, natural resistance-associated macrophage protein 1 (NRAMP1),[84] no association with NRAMP1 genotype and leprosy was found in one study.[88] This study did demonstrate an association with vitamin D receptor genotype. Another study suggests that genetic control of cytokine production may be the key.[89] This is an important and complex field and additional findings will probably contribute greatly to our understanding of the epidemiology of leprosy.

6. Mechanisms and Routes of Transmission

Over the years, the oldest and most prevalent opinion was that the major route by which *M. leprae* gained access to the human host was through the skin. This could have resulted from direct skin contact with an infectious case or from introduction of the organism onto the skin by a fomite. Evidence cited[58] to support the theory of infection by direct skin contact includes the appearance of the first leprosy lesion in children at the sites most touched by their infected mothers, accidental inoculation by tattooing or at surgery, and inoculation via thorn penetration in animal models.[90] This evidence actually provides relatively little support for transmission via the skin. While the bacillus might occasionally be inoculated via injuries that pierce the skin, a history of such an injury is rare and the literature contains a number of studies showing that the site of the first lesion in children is not related to the extent to which an area is touched.[91] Furthermore, over 50% of cases in regions of low to moderate endemicity give no history of contact with a leprosy case.[92] Thus, "prolonged" or "intimate" contact in these instances seems unlikely. While it is possible that fomites have contact with *M. leprae* in soil or peat and transfer it to skin of a person, there is no clinical evidence to support this route of acquisition. In addition, it seems unlikely that *M. leprae* could penetrate normal healthy skin. Thus, only individuals with an open wound or with dermatitis would be susceptible.

The possible role of the nose and upper respiratory tract in the transmission of leprosy has been studied extensively. Pedley[93] demonstrated that although bacilli may easily be found in the nasal discharge, they are difficult to locate on the skin surface. Davey and Rees[94] demonstrated that a high percentage of bacilli that are passed out in the nasal discharge are viable and can survive up to 7 days. A single nose blow from each of 31 patients gave a mean discharge of 1.1×10^8 bacilli with a mean morphological index (MI) of 16.9%. Studies have also shown that thymectomized mice,[95] nude mice,[96] and immunocompetent mice[97] may be infected with *M. leprae* via the respiratory route and that dissemination of the infection follows this mode of infection.[95] Job summarized experimental data in mice that demonstrate that nasal mucosa and abraded skin over the cooler parts of the body are the preferred sites of entry of *M. leprae*.[98] It is thus highly likely that most cases of leprosy are contracted through the respiratory route. One major difference from tuberculosis is that *M. leprae* gains access via the upper respiratory mucosa, primarily the nose, while *M. tuberculosis* must be inhaled into the alveoli of the lung to establish infection.

A third proposed route of infection is transmission of the leprosy bacilli by insect inoculation, as opposed to

fomites simply depositing the bacilli on the skin. Reports of acid-fast bacilli in the stomachs of insects which had bitten leprosy patients appeared as early as 1912.[60] Although the theoretical risk of transmission by insects was supported by the demonstration that mosquitoes (*Aedes aegypti*) took up *M. leprae* when biting untreated patients and were capable of transmitting the infection to mouse footpads,[99] epidemiologic studies have not shown any correlation between mosquito or other insect bites and the occurrence of leprosy. These strong epidemiologic data indicate that the theoretical possibility mentioned above is not important in the epidemiology of this disease.

Several other possible sources of infection have been suggested. Beginning in 1975, it was reported that armadillos caught in the wild in Louisiana were infected with acid-fast bacilli that ultimately proved to be *M. leprae*.[100] Numerous other surveys have confirmed that this occurs in 2–6% of armadillos caught in parts of Texas and Louisiana.[101] The significance is uncertain and the problem appears to be confined to the United States and Mexico. Originally it was felt that this was not a likely source of human infection,[102] but some investigators[103,104] have found evidence that transmission of the disease from armadillos to humans may at least occasionally occur. Leprosy has also been found in a chimpanzee from Sierra Leone[105] and a sooty mangabey monkey from Nigeria. These animals might have caught it from handlers, but its apparent transmission to a second mangabey monkey housed with the first indicates that monkey-to-monkey spread can occur.[106] Evidence that *M. leprae* may exist free in nature has also been found,[107] raising the possibility that this may be the source of infection in these various animals and perhaps occasionally in man.

Perhaps of greater importance than all of these are epidemiological data[23] that show a relatively high incidence of *M. leprae* in nasal swabs from individuals in highly endemic areas with no evidence of leprosy. There are not sufficient data yet to know whether or not these carriers transmit the organism to others, how often this occurs if it does occur, and what proportion of carriers develop clinical leprosy.[23]

7. Pathogenesis and Immunity

Although the precise route of infection by *M. leprae* is unknown, early lesions are observed in the oro- and nasopharynx and in the skin over cool regions of the body, including the forehead and malar areas of the face. Destructive lesions of the nasopharynx, larynx, and hard and soft palate are well documented[108] but, since the introduction of effective antimicrobial therapy, they are seldom seen, and the organs that are of greatest concern today are the skin and peripheral nerves.

The pathogenesis of leprosy in these tissues is dependent upon the immunological capability of the individual host to develop effective cellular immunity (CMI) to *M. leprae*. Abundant antibody is produced in the lepromatous forms of the disease but does not provide any known benefit to the patient. Cross-reactive antibodies in leprosy sera may give false-positive results in serologic tests for HIV and other pathogens, however.[109]

Among those who are susceptible, the immunopathogenesis of leprosy proceeds in two phases, mediated first by mechanisms of innate and then of specific acquired immunity. Substantial evidence now indicates that genes operating at two different levels influence resistance to *M. leprae* in man. By positional cloning, a locus associated with overall susceptibility to *M. leprae* has been identified in the PARKIN/PACRG gene.[110] This codes for a ligase in the ubiquitin proteasome pathway and may modulate innate immunity to *M. leprae* through influences on processing and presentation of *M. leprae* antigens by macrophages and dendritic cells.[111] Other studies have identified an association of leprosy susceptibility with the NRAMP gene,[112] which influences transport of iron and other divalent cations across the phagosomal membrane.[113] In Section 5.11, we referenced both positive and negative data with regard to an association with the NRAMP gene. It is not clear whether these apparently conflicting results are related to differences in methodology, study designs, study populations, or to other factors.

Several additional genes have been associated with the development of different degrees of CMI to *M. leprae*, ranging from total inability to eliminate organisms at one extreme, termed lepromatous, to the ability to develop well-formed epithelioid granulomas at the other extreme, termed tuberculoid. (The clinical and histopathologic features of this uniquely broad immunopathologic spectrum are described below, Section 8, *Patterns of Host Response*.) These genes, which appear to be expressed through T-cell-mediated influences on CMI in leprosy,[114] have been reviewed recently[42] and include genes for MHC class II molecules (chromosome 10p13),[115] transporter associated with antigen processing (TAP),[116] and tumor necrosis factor alpha (TNF-α).[117,118] Macrophages (MΦ) and dendritic cells bind *M. leprae* by means of several receptors, including C-type lectins and toll-like receptors (reviewed in reference 42), and the organism is ingested into phagosomes. *M. leprae*-infected MΦ remain remarkably functional in some respects, releasing the pro-inflammatory cytokine tumor necrosis factor-α, for example, but they also release the inhibitory molecule prostaglandin E_2 and are refractory to activation by interferon-γ.[119]

Following binding of *M. leprae* to TLR-2, MΦ develop enhanced phagocytic capability, reflected by the enormous numbers of bacilli seen in lepromatous lesions. In the appropriate genetic context, they may develop further into dendritic cells with enhanced antigen-presenting capability; such cells are observed in tuberculoid lesions.[120]

After presentation of *M. leprae* antigens to T-cells and secretion of interleukin-2 (IL-2), the immune response within lesions diverges generally along two different cytokine pathways according to the different types of leprosy. Patients unable to generate sufficient CMI produce TH-2 cytokines, while those capable of developing a granulomatous response display a TH-1 cytokine profile.[121,122] The understanding of the CMI response to *M. leprae* is incomplete, and the mechanisms underlying the selection of either pathway in individual patients remain to be elucidated (reviewed in reference 42). Nor is the TH-1/TH-2 paradigm strictly observed in leprosy; in some cases a TH-0 pattern is observed.[123] A major difficulty in interpreting these human studies is an inability to determine or control for the differing duration of illness in different patients.

Immunohistological examination of cutaneous lesions reveals a predominance of CD4+ cells over CD8+ cells in tuberculoid lesions, associated with the organization of the infiltrate into epithelioid granulomas. In contrast, in lepromatous lesions, the ratio of CD4+:CD8+ cells is ≤ 1, and both types of T-cells are randomly distributed throughout the infiltrate.

Within MΦ, reactive nitrogen products kill *M. leprae*, and MΦ capable of such killing show increased expression of inducible nitrogen oxide synthase (iNOS).[124] Reactive oxygen intermediates, capable of killing *M. tuberculosis*, do not appear to harm *M. leprae*.

M. leprae is the only bacterial pathogen of man that is capable of invasive infection of peripheral nerves, and involvement of peripheral nerves is the sine qua non of leprosy. Infection of cutaneous nerves occurs very early in the disease, since hypoesthesia or anesthesia of lesions is one of the clinically diagnostic features of early lesions. Animal models suggest that *M. leprae* initially localizes on the surface of peripheral nerves, possibly as a result of lymphatic drainage from the skin, and subsequently follows the vascular supply to gain access to the endoneurial compartment of the nerve.[125]

Within the nerve, *M. leprae* cell wall molecules bind to Schwann cells via laminin receptors, including alpha-dystroglycan,[126] and are ingested by Schwann cells. Viable *M. leprae* appear to be able to inhibit acidification of phagocytic vesicles.[127] Schwann cells in vitro do not kill *M. leprae*, and in vivo they probably offer a comfortable environment within which these bacilli are able to proliferate and survive for long periods of time.[127] Early studies also suggest that the bacilli have minimal direct effect on Schwann cell gene expression and function,[128] but infected Schwann cells may be targets of immunologically mediated injury.[129]

Perineural inflammation is characteristic of leprosy, possibly reflecting the initial localization of bacilli to the surface of the nerve. The composition of the mononuclear cell infiltrates found within nerves is similar to that seen in the skin. Neuropathic pain in leprosy has only recently been appreciated and is now being studied[130] but, paradoxically, it is not nearly as frequent as might be expected from the extent of peri- and intraneural inflammation observed.

Sensitive methods for measuring functional nerve impairment have demonstrated that lepromatous patients, with focal, heavy infection of nerves, experience subtle sensory loss earlier than do tuberculoid patients,[131] although patients seeking medical attention for sensory and motor deficits are more often tuberculoid patients, in whom the severity of granulomatous inflammation apparently accelerates nerve damage more rapidly than the ineffective inflammatory response in lepromatous disease. The mechanisms of neuropathy in leprosy are poorly understood but are currently subjects of intensive investigation. Evidence to date indicates that the same immunological and inflammatory processes observed in the skin occur also in nerves, to the detriment of Schwann cells and axons.[132] Ultimately, untreated *M. leprae* infection of nerves results in demyelination and subsequent nerve conduction abnormalities in all types of the disease.[133] If untreated, this may result in permanent, profound anesthesia, paralysis, and deformity, and in autonomic neuropathy with absorption of the bones of affected hands or feet.

Neuropathy affecting the eyes deserves special note, as pointed out by Daniel et al.[134] For example, corneal nerve involvement may lead to decreased sensation, corneal abrasion, and blindness. Impaired function of the peri-orbital musculature may result in lagophthalmos.

8. Patterns of Host Response

Leprosy presents a wide range of clinical and histopathologic manifestations that puzzled and frustrated understanding of the disease until it was appreciated that this diversity was based on the ability of the host to develop a cellular immune response to *M. leprae*. Skinsnes proposed the first full formulation of the concept of an immunopathologic spectrum of leprosy in 1964,[12] and Ridley and Jopling presented a practical classification scheme based on this concept in 1966.[135] This classification enabled a reasonable

degree of global uniformity and comparison in clinical practice, and the underlying theory has become the yardstick against which new findings concerning the immunology of leprosy are measured.

Very early lesions may present histologically as relatively non-specific perineural infiltrates in which rare acid-fast bacilli can be demonstrated, but without sufficient infiltrates to classify them these are called "indeterminate" (Figure 1). Care should be taken to use this term only when the biopsy shows diagnostic evidence of leprosy (nerve involvement and acid-fast bacilli), since a diagnosis of leprosy often has significant impact on the patient's mental and physical health, social status, and employment and also impacts the patient's family.

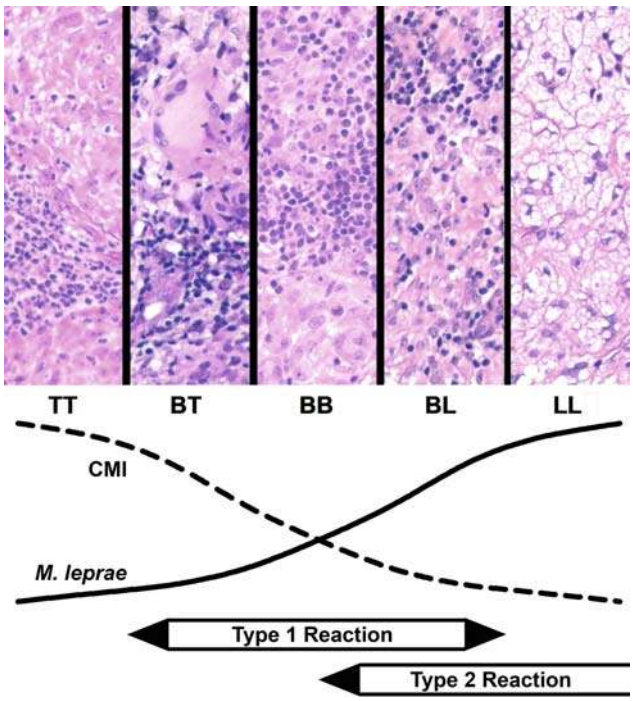


Figure 1. The immunopathologic spectrum of leprosy. Representative sections of human skin biopsy specimens illustrate the full spectrum of inflammation seen in leprosy, from the well-formed granulomatous inflammation of polar tuberculoid (TT) lesions to the completely disorganized sheets of foamy macrophages observed in polar lepromatous (LL) disease. Most patients are classified in the broad borderline region, which is further divided into borderline tuberculoid (BT), mid-borderline (BB), and borderline lepromatous (BL). As indicated in the diagram, the number of bacilli present in lesions (*solid line*) varies from rare in TT lesions to abundant in LL ones. This is inversely related to the patient's degree of cell-mediated immunity (CMI) to *M. leprae*. The *open arrows* indicate the portions of the spectrum in which patients are at risk for type 1 ("reversal") or type 2 ("*erythema nodosum leprosum*") leprosy reactions.

Among patients with established lesions,the five-part Ridley–Jopling classification identifies, at one extreme, patients with a high degree of cell-mediated immunity and delayed hypersensitivity, presenting with a single (usually), well-demarcated lesion with central hypopigmentation and hypoesthesia. Biopsy of such a lesion reveals well-developed granulomatous inflammation and rare acid-fast bacilli demonstrable in the tissues; this is termed polar tuberculoid (TT) leprosy (Figure 1). Unlike tuberculosis, caseous necrosis is rare in TT leprosy granulomas. At the other extreme, in patients who have no apparent resistance to *M. leprae*, biopsy of a raised or nodular skin lesion reveals sheets of foamy macrophages in the dermis, containing very large numbers of bacilli and micro-colonies called "globi". This non-resistant, highly infected form of the disease is termed polar lepromatous leprosy (LL). The majority of patients, however, fall into a broad "borderline" category between these two polar forms; this is sub-divided into borderline lepromatous (BL) leprosy, mid-borderline (BB) leprosy, and borderline tuberculoid (BT) leprosy, each with a correspondingly graduated bacterial load and organization of the inflammatory infiltrate (Figure 1). In the World Health Organization's simplified clinical classification scheme, TT and BT types are generally identified as "paucibacillary" (PB), and LL, BL, and BB types as "multibacillary" (MB), though there is not one-to-one correlation between the two classifications.

Overall, the majority of patients are classified in the LL, BL, or BT categories, but the distribution of patients within the entire spectrum varies according to racial background (e.g., TT and BT types are more common in African populations, and LL and BL types are more common in Caucasian populations). Patients in the two polar groups (LL and TT) are relatively stable in their immunopathologic response to *M. leprae*, but considerable shifts in immunological and clinical status can be observed in patients in the borderline portion of the spectrum. Current understanding of the genetic and immunologic basis for this broad range of human immune response to *M. leprae* has been discussed above (Immunity and Pathogenesis).

"Reactions" are acute inflammatory complications often presenting as medical emergencies during the course of treated or untreated HD. Two major clinical types of leprosy reactions occur; together they may affect 30–50% of all leprosy patients.[136,137,138] Because *M. leprae* infects peripheral nerves, the inflammation associated with reactions is often a medical emergency, as severe nerve injury may develop rapidly, with subsequent loss of sensation, paralysis, and deformity.

The cause(s), mechanisms, and treatment of these reactions remain highly problematic, both for clinicians and basic

scientists.[42] The different types of reactions appear to have different underlying immunologic mechanisms, but these are poorly understood, in spite of a substantial body of detailed descriptive information, and the factors that initiate them are unknown.

Type 1 reactions occur in patients in the immunologically labile, borderline portion of the spectrum (i.e., BL, BB, and BT). They are also known as "reversal" reactions, because clinical and histopathologic observations suggest that the cell-mediated immunity (CMI) in the lesions had increased ("upgrading") or decreased ("downgrading").[139] The increase in cell-mediated immunity against *M. leprae* is the underlying mechanism for the type 1 reaction and is most often seen after institution of effective therapy against *M. leprae*. There are other situations in which such an increase in cell-mediated immunity to *M. leprae* may occur, however.

Those experienced in caring for patients with tuberculosis are familiar with the clinical situation of radiologic progression of pulmonary tuberculosis with mild clinical symptoms in a patient receiving systemic corticosteroid therapy. When the corticosteroid therapy is stopped or the dose of corticosteroid markedly reduced, the patient may develop severe clinical symptoms due to an increase in CMI. The same may occur in leprosy, either in a patient already receiving corticosteroids when leprosy is diagnosed or in a patient with leprosy receiving corticosteroids when the dose is reduced.

Normal pregnancy is associated with a progressive suppression of cell-mediated immunity.[140] The increase in cell-mediated immunity in the postpartum state is almost certainly responsible for the postpartum type 1 reactions reported in the review by Lockwood and Sinha[141] and diagnosed in a number of patients by one of us (RIF).

The recently recognized clinical entity called immune reconstitution inflammatory syndrome (IRIS) has been reported in a number of patients with tuberculosis where the clinical manifestations of tuberculosis became more severe when the patient's CD4+ T-cell count and cell-mediated immunity increased after institution of highly active antiretroviral therapy (HAART). Although early studies did not show a significant effect of HIV on the epidemiology or clinical manifestations of leprosy, subsequently IRIS has been recognized in patients who have both AIDS and leprosy in endemic countries[77,78,109] and more recently in the United States.[142] Trindade and colleagues recently reported two patients with leprosy and HIV infection who developed type 1 reactions prior to the administration of HAART.[143] Ustianowski and coworkers reviewed the current knowledge of the interactions between HIV and leprosy in another recent paper.[144] The clinical findings and immunologic changes in leprosy-associated IRIS are identical to type 1 reactions seen in patients without HIV. The difference is that the type 1 reaction in IRIS follows an increase in CD4+ T-cells in response to HAART given prior to or concomitant with the development of the type 1 reaction.

Finally, Scollard et al. reported two patients with leprosy that became clinically apparent after treatment with infliximab, a human monoclonal antibody that binds to tumor necrosis factor-α.[145] Both patients developed type 1 reactions after discontinuation of infliximab, presumably due to restoration of cell-mediated immunity. Infliximab and other tumor necrosis factor-α inhibitors interfere with the cell-mediated immune response to mycobacteria, and treatment with these agents may precipitate clinical leprosy or tuberculosis in patients who have latent infection with *M. leprae* or *M. tuberculosis*, respectively.

These reactions often develop gradually and their natural course may last for many weeks or months. They may develop before or during the course of treatment, presenting as induration and erythema of pre-existing lesions (although new lesions may also appear), frequently with prominent acral edema, and often with progressive neuritis. They may also occur after treatment has been completed, presumably because *M. leprae* antigens remain in the body for a prolonged period even after successful treatment. Post-treatment type 1 reactions have been noted most often in patients treated with the 6-month PB regimen. It is likely to be seen more often in patients treated with the MB regimen in the future if treatment is given for 12 months rather than 24 months. In severe reactions the lesions may ulcerate. Cellular immunity to *M. leprae* is spontaneously enhanced in "upgrading" reactions, with increased levels of TH-1 cytokines in cutaneous lesions[123,146] (reviewed in reference 42). Corticosteroid treatment generally controls the inflammation in type 1 reactions, but management is empirical because no laboratory tests are available to determine when the underlying immunologic activity has ceased. Occasional patients may require very high doses of corticosteroids and rarely other drugs are necessary.[147]

Type 2 reactions, also known as *erythema nodosum leprosum* (ENL), occur in LL and BL patients who have poor cellular immunity to *M. leprae*, abundant bacilli (i.e., antigen) in skin and nerve lesions, and high levels of circulating immunoglobulins.

These patients experience an abrupt onset of crops of very tender, erythematous nodules that may develop on the face, extremities, or trunk, without predilection for pre-existing lesions. Systemically, these patients often also experience fever, malaise, and painful neuritis. Iridocyclitis and episcleritis, orchitis, arthritis, and myositis may also accompany this reaction. In severe type 2 reactions some of the

cutaneous lesions may ulcerate. The usual course of an untreated episode of a type 2 reaction is 1–2 weeks, but many patients experience multiple recurrences over several months.

The acute lesions of a type 2 reaction are characterized by a neutrophilic infiltrate superimposed upon a chronic lepromatous pattern, observations that have long dominated thinking about this reaction. Although widely regarded as an immune-complex phenomenon,[148] the evidence is not entirely convincing and some findings implicate cellular immunity in type 2 reactions,[123] reviewed in reference 42. Interestingly, attempted therapy of lepromatous leprosy by intra-lesional injection of IFN-γ elicited type 2 reactions in some patients.[149]

For type 2 reactions, thalidomide is the most efficacious drug.[150] (For decades, the type 2 reaction was the primary indication for the use of thalidomide, and the drug might well have been forgotten entirely had it not been so valuable for the treatment of this complication of leprosy.) The use of this drug is very strictly regulated in the United States because of its teratogenicity, and thus it is never the first line of treatment in this country, in spite of its efficacy. Therefore, corticosteroids are the most widely used agents for the treatment of these reactions in the United States.[151,152] In some countries, however, thalidomide is the agent of choice. The anti-inflammatory properties of clofazimine may also be useful in treating type 2 reactions. Antimicrobial therapy should be maintained throughout treatment of both types of reactions.

The Lucio phenomenon is an acute, severe, necrotizing vasculopathy occurring primarily in patients of Mexican ancestry.[153] Fortunately this complication is rare, because it is associated with high morbidity and mortality. These reactions have been associated with the presence of high levels of cryoglobulins, and *M. leprae* antigens have been associated with some of these,[154,155] but the role they might play in these reactions is unclear. Characteristically, endothelial cells are unusually heavily infected and may be seen in various stages of degeneration. Lucio reactions may be accompanied by a profound anemia, and severe cases may require intensive inpatient treatment, with debridement and wound care comparable to the management of severe burns.

9. Control and Prevention

9.1. General Concepts

Leprosy control today is based mainly on case detection and treatment. The introduction of rifampin, discovered in 1968[156] and first used for the treatment of leprosy in 1970,[157] revolutionized treatment. Rifampin is the most potent anti-*M. leprae* drug in clinical usage. It kills 99.9+% of all viable *M. leprae* within a few days. Since treated cases are thereby apparently rapidly rendered noninfectious, isolation is not required nor is hospitalization necessary for administration of antimicrobial therapy. The success of this approach, of course, depends on having an effective leprosy control program that can be either vertical or horizontal. As leprosy has become less common, horizontal programs that are integrated into the primary health care system have become more common. Guidelines for all aspects of control programs are contained in a number of the WHO publications,[14,158,159] but obviously all such guidelines must be adapted to the individual country situation. Special challenges include non-specific presentation of the disease, lack of familiarity of health care workers with the disease, stigma that makes patients and their families reluctant to report for diagnosis, and geographic barriers, all of which impede early case detection. While there is effective treatment, a major challenge is ensuring compliance with multiple drugs over a prolonged period of time. Although those working in leprosy may view today's regimens as short in comparison with previous regimens, patients do not view them as short.

Case detection has been based mainly on the examination of household contacts and the awareness of health care providers of the early symptoms and signs of leprosy. In recent years, however, it has become increasingly common that no household case or other known source case is detected. General population surveys and screening of school children or other special population groups are generally insufficiently productive in terms of detection of new cases to justify their cost. Exceptions may include areas with a high prevalence rate, particularly where the population has relatively poor access to medical care and/or the control program is just being developed, and patients previously treated with dapsone monotherapy, in whom the relapse rate is known to be high. Programmatic success is measured by detection of disease at a relatively early stage (indicating an effective program of case detection), a low disability rate in new cases (another indication of effective and early case detection), the percentage of patients who have completed treatment (indicating an effective treatment program), and a low number of cases in children (indicating limited ongoing transmission of disease). In the United States, with abundant resources and a low number of cases, the goals should clearly be curative treatment of every patient and prevention of any disability not already present at the time of diagnosis.

9.2. Antimicrobial Chemotherapy of Leprosy

Dapsone, first used successfully in 1941,[160] was the mainstay of treatment for leprosy for almost 40 years.

Although clofazimine was introduced for leprosy treatment in 1961[161] and rifampin in 1972,[162] and multidrug therapy was proven to be effective in a study in 1972,[163] dapsone monotherapy was the standard regimen from the time of its introduction until the 1980s. This is both remarkable and unfortunate. Streptomycin, the first effective antituberculosis chemotherapeutic agent, was discovered by Waksman's group in 1943[164] and first used to treat tuberculosis in 1948.[165] It soon became apparent that monotherapy of tuberculosis leads to resistance to the antimicrobial agent and that multidrug therapy is essential.[166] This has been the paradigm for treatment of tuberculosis ever since. It took nearly four decades for this concept to be applied to leprosy. Part of the reason was the inability to culture *M. leprae* and to study drug resistance. Dapsone resistance was first demonstrated in the mouse footpad in 1964.[167]

Multidrug therapy is now applied universally in leprosy control. By 1981, primary and secondary dapsone resistance had been demonstrated, with resistance rates as high as 19% in some groups of multibacillary patients with relapse.[168] A WHO Expert Committee recommended in 1981 that all patients be treated with multidrug therapy.[168] WHO developed a field classification, categorizing patients as either paucibacillary (PB) or multibacillary (MB). Though some changes have been made in the criteria for these two classes, the basic classification remains in effect, as do the treatment regimens recommended by the Expert Committee. The PB regimen consists of rifampin 600 mg, given once each month under direct supervision for 6 months, and dapsone 100 mg daily, taken by the patient without supervision for 6 months. The regimen for MB disease includes the same treatment as the PB regimen with two changes that increase the potency of therapy. First, patients also receive clofazimine, given in a dose of 300 mg monthly at the same time as the supervised rifampin dose and 50 mg taken daily with the dapsone without supervision. Second, the duration of treatment is extended to 24 months. Although the regimen that was developed in 1981 and has been only slightly modified subsequently has been administered to millions of patients, there has never been a randomized controlled trial of its efficacy. Nevertheless, its efficacy has been demonstrated by relapse rates in the range of 1% for either regimen with prolonged follow-up. Assessing the true relapse rate is difficult, as some of the PB relapses almost certainly reflect immunologic phenomena (reactions) occurring after the relatively brief course of treatment. In addition, there is no diagnostic test to differentiate relapse from re-infection. Thus, the true relapse rate is probably well below 1% with 15–20 years of follow-up. There are now data to indicate one major exception to the preceding statements regarding the efficacy of the WHO regimens. Studies strongly suggest that MB patients with very high bacillary loads (BI of $\geq$4+) have a much higher relapse rate.[169,170] Given the increased relapse rate in patients with a high bacillary load, the very slow growth of *M. leprae*, and the difficulty of detecting relapse at an early stage, it is possible and perhaps likely that relapse rates will be higher with longer periods of follow-up.

The standard approach to therapy of patients who relapse after completion of the MB regimen has been retreatment with the same regimen, as drug resistance does not seem to be a major factor in these relapses. The authors prefer increasing the potency of the regimen in the initial treatment of these high-risk patients, either by giving rifampin on a daily rather than monthly basis or by extending the duration of treatment beyond 24 months, or both, and to similarly increase the potency in patients who are retreated for relapse. There are no data, however, to tell the clinician that one or another of these three options is the best choice.

WHO has made two relatively recent changes to the standard multidrug regimen. The first relates to the MB regimen. With regard to treatment of MB disease, analysis of patients who dropped out of treatment without completing the full 24 months revealed that those who had completed at least 12 months of treatment had a much lower relapse rate than those who had completed less than 12 months of treatment. WHO stated that "it is possible that the duration of the current MDT regimen for MB leprosy could be shortened further to 12 months"[171] and now recommends 12 months of treatment with the MB regimen as the standard.[172] In view of the success of the 24-month regimen and the limited experience with the 12-month regimen, the authors recommend the 24-month regimen whenever resources permit this. This clearly applies to the United States.

The second WHO change relates to patients presenting with a single skin lesion. Because some clinical data suggest that such patients have a high rate of spontaneous cure and a low rate of relapse, WHO now recommends treatment of these patients with a regimen called ROM, which stands for rifampin, ofloxacin, and minocycline.[173] The treatment consists of single doses of rifampin 600 mg, ofloxacin 300 mg, and minocycline 100 mg, given together. Again, there have been no randomized controlled studies of this regimen. Limited data suggest that it is effective,[173,174] but there have been no large studies to compare this to other regimens and the analysis of the data has been questioned.[175] In the United States, its use should be restricted to those very few cases where compliance, drug intolerance, travel, or some other factors make it the preferred regimen.

While dapsone, rifampin, and clofazimine have been the standard drugs for more than two decades, the ROM regimen above reflects the interest in, desire for, and availability of other agents. Aside from the use of ofloxacin in the ROM

regimen, ofloxacin and other fluoroquinolones have been studied in mice[176] and humans.[177] There are also limited data concerning the effect of minocycline on *M. leprae* in humans.[178] Clarithromycin is also active against *M. leprae* and has been studied to a limited extent in humans.[179] This and other potential new drugs have been studied in animals.[180] All of these drugs are more expensive than the older agents. The cost factor could be overcome if the duration of treatment could be dramatically shortened, as with the ROM protocol. This would also have the advantage of reducing problems with compliance and reducing program costs incurred in maintaining patients on the registry and on treatment for a long period of time. Another problem, however, is that the falling incidence and prevalence of leprosy make it unlikely that there will be sufficient numbers of patients treated with these agents to determine their relative efficacy in comparison with the standard regimens.

9.3. Follow-Up after Antimicrobial Therapy

Because of the high cure rate with WHO MDT, WHO does not recommend routine follow-up of patients who have completed therapy. Rather, emphasis is put on education of the patient regarding symptoms and signs of relapse or reaction.[158] In countries with sufficient resources, programs may chose to see patients periodically following completion of treatment, both to assess the patient for relapse or reaction and to reinforce patient education.

9.4. Follow-Up of Contacts

WHO recommends[158] an evaluation of household contacts only at the time of diagnosis of the index case. Thereafter, contacts are advised to return only if signs or symptoms of the disease occur. Nonetheless, many control programs and the United States' recommendations are for annual follow-up examinations of contacts for 5 years. Early detection among contacts or the general population obviously depends on having physicians and other health practitioners who are trained to recognize the early symptoms and signs of the disease. As a result of efforts to eliminate leprosy as a public health problem by the year 2000, there has been significant improvement in this regard in most leprosy-endemic countries in recent years. Nonetheless, the effort must continue even after that goal is reached if we are to avoid a future resurgence of leprosy. The clinical examination to evaluate contacts for symptoms and signs of leprosy is equally important as it provides an opportunity to instruct the contact regarding these. Because of the variable "incubation period", patients may develop leprosy after they have completed their evaluation period or between periodic evaluations. Patients who have been properly educated will know when and how to get assistance if they are concerned, either after they have been released from the contact evaluation program or in between yearly examinations.

In the United States, the goal should be early detection and appropriate treatment of all patients. Because of the limited extent of the problem in this country, however, only household contacts or other very close contacts need to be examined on a regular basis. In selected circumstances, it may be beneficial to broaden the circle of contacts that is examined. For example, in Hawai'i, detection of a single case in an immigrant from the Republic of the Marshall Islands led to detection of a number of other cases. Subsequent active voluntary screening of many Marshall Islanders in Hawai'i led to detection of a number of additional cases.[19] If such community screening is done, it must be done with great sensitivity and with the assistance of leaders of the community undergoing screening. If these factors are ignored, the screening process is likely to be rejected by the community and members of that community may be subject to great stigma. It is doubtful that we can eliminate the disease in the United States, since most new cases occur among immigrants or aliens, but the problem is readily controllable.

Health education of patients and their families cannot receive too much emphasis, either in the United States or elsewhere. Patients will require antimicrobial treatment for 6 months to 2 years. Patients with disability, reactions, or other complications may require prolonged or lifelong care. As noted above, education is essential to ensure the cooperation of patients and their families and close contacts and to minimize stigma.

9.5. Chemotherapeutic Approaches to Prophylaxis

Chemoprophylaxis as a leprosy control measure has been attempted in a number of studies over the years since sulfone drugs were first found to be effective for the treatment of leprosy in the 1940s. Investigators reported a 40–80% success rate using dapsone.[181] Unfortunately, most had insufficient follow-up to determine whether the disease was actually prevented or only delayed in those contacts destined to develop MB disease. The best trial in this regard was carried out on the islands of Pohnpei and Pingelap in what is now Pohnpei State in the Federated States of Micronesia, which at the time of the study had a case detection rate of 7 per 1,000.[182] A relatively successful attempt was made to treat all of the population with 15 injections of acedapsone over a 3-year period. This drug is the diacetyl derivative of dapsone and, given in a dose of 225 mg every 11 weeks, maintains a low, relatively stable level of dapsone in the blood throughout the interval. As expected, the incidence of

new cases fell to zero during the trial, but cases began appearing again shortly after the trial was discontinued and continued to appear for some time. Even if acedapsone prophylaxis had been successful, the prolonged period of time it had to be given would have markedly limited its usefulness under field conditions.

A trial of a single large dose of rifampin (25 mg/kg in adults) to prevent leprosy[183] demonstrated some effectiveness but the number of patients was too small to clearly define the benefits from this approach. More recently, WHO tried two other approaches in Pacific Island populations with a high incidence and prevalence of disease. In one, they attempted to give a single dose of ROM to every citizen. In the second, they treated all known contacts of cases with the PB regimen. There are presently no data to evaluate the success of either of these approaches.[184,185] Thus, WHO at this time does not recommend any form of chemoprophylaxis to prevent leprosy.[158] In the United States, the question of prophylaxis arises occasionally, most often when a parent of a young child develops MB disease and the treating physician considers preventive treatment for the child. In most instances, as the degree of contagiousness decreases rapidly once the parent is started on treatment, it is sufficient to treat the patient and screen the child periodically. Were one to institute preventive treatment, one would have to either use the PB regimen or the ROM regimen, starting only after the parent has had sufficient treatment to permanently interrupt transmission to the child. There are no data to guide the physician regarding the duration of such preventive therapy.

9.6. Immunization

Over the years, there has been a great deal of interest in immunization to prevent leprosy. Although no specific antileprosy vaccine is yet available, BCG offers about 50–80% protection against leprosy[186,187,188] and is more effective for leprosy than it is for tuberculosis. Trials using vaccines containing two cultivable mycobacteria, *Mycobacterium w* and the ICRC bacillus, as well as heat-killed *M. leprae*, with or without simultaneous administration of BCG, showed that each of these vaccines may have some effect in preventing leprosy[188,189,190] but, given the apparent effectiveness of the current approach emphasizing case detection and curative antimicrobial therapy, it seems unlikely that they will play a significant role in global leprosy control. In addition to preventing leprosy, there is evidence that these vaccines may also have an immunotherapeutic effect on this disease. Probably related to this immunotherapeutic property, a number of studies in India have suggested that these mycobacterial vaccines might shorten the duration of MDT necessary for cure. Again, given the effectiveness of current treatment regimens and the diminishing number of patients available to participate in clinical trials, it seems unlikely that this will play a significant role in the years ahead. Nevertheless, interest in vaccine development continues. Investigators are currently using molecular approaches and attempting to develop a multipurpose vaccine that could provide protection against several diseases including leprosy.[81]

10. Unresolved Problems

Although there was failure on a global level to reach the WHO goal of elimination of leprosy as a public health problem (prevalence less than 1 per 10,000 population) by the year 2000, great progress has been made in reducing the prevalence and incidence of leprosy. The tools to continue to reduce and contain this disease are clearly at hand. The crucial factors in the years to come in terms of containment are likely to be continued action on the part of governments and non-governmental organizations to maintain leprosy control programs in the face of competition for funds from other public health problems, including infectious diseases such as the acquired immunodeficiency syndrome (AIDS), tuberculosis, malaria, and influenza, both human and avian. Specialized facilities and services have been or are being integrated into general medical services. This integration and the accompanying decrease in the number of clinicians with experience with and interest in leprosy lead to both underdiagnosis and overdiagnosis and difficulty in diagnosing new cases at an early stage. There are concomitant problems with management of acute and chronic complications of leprosy. In addition, although WHO does not include them in the number of cases of leprosy, there are many patients with severe leprosy-related disabilities who will continue to need various services, including medical care, rehabilitation, job training, and social and mental support, from control programs.

In spite of the tremendous progress that has been made in leprosy control, made possible in part by a well-funded, globally adopted WHO program, the failure to reach elimination goals in all countries and in parts of other countries reflects the pitfalls in relying solely on antimicrobial therapy for elimination.[191,192] While there have been major advances in understanding the immunologic aspects of leprosy, these changes have not yet led to the development of an effective vaccine. If such a vaccine should become available, this will require challenging decisions, such as what is an acceptable dollar cost for the vaccine, what are the costs of implementation of a vaccination program, and which populations and individuals should receive the

vaccine? A possible strategy is to develop a multivalent vaccine that would be effective for several diseases affecting populations also affected by leprosy, such as tuberculosis and malaria. This approach is in the early experimental stages of development.

While remarkable technical advances enable us to describe many immunological aspects of leprosy and its broad immunopathological spectrum at the molecular level, we still cannot explain the mechanisms that determine whether or not an individual is susceptible to developing clinical leprosy and, if susceptible, what degree of susceptibility is present. In addition, understanding of these basic mechanisms will be important in assisting clinicians in managing immunological reactions and in managing neuritis.

Treatment of leprosy immunologic reactions continues to be a challenging and important aspect of overall patient care, since reactive episodes may result in worsening of existing disability or development of new disabilities. Corticosteroids and thalidomide are, for practical purposes, the only available therapy. While both are reasonably effective for the proper indication, toxicity is a major problem with corticosteroids. Toxicity can also be a major problem with thalidomide, and the potential for teratogenicity limits the availability of this drug. It would clearly be a major breakthrough to have pharmacologic agents that are safer and at least as effective. In addition, diagnostic tests for leprosy reactions will be valuable in determining when to institute therapy and in guiding the clinician with regard to duration of therapy or dose of the agent(s) used.

The mechanisms of nerve injury in leprosy are still poorly understood, hampering more effective prevention and treatment. Nerve biopsy of seriously affected nerves is usually impossible because of the ensuing permanent loss of nerve function. Thus, most current knowledge of the processes involved has been derived indirectly, primarily from studies of Schwann cells in vitro. Although the armadillo appears to offer a good experimental model, at least in terms of the patient with lepromatous leprosy, exploitation of this model has been severely hampered by the lack of reagents for immunological studies of this animal. Successful conclusion of research to sequence the armadillo genome should offer new possibilities for determining the mechanisms involved in the pathogenesis of neuritis in leprosy.[193] Successful prevention or reversal of the peripheral neuropathy of leprosy would dramatically reduce the medical, psychological, social, and economic impacts of infection by *M. leprae*.

Better understanding of the biology of *M. leprae* and of the response it elicits in humans is required for the development of a vaccine. Although *M. leprae* still cannot be cultivated, the availability of the complete genome and related molecular discoveries should in time provide answers to a variety of questions. Comparison of the genome of *M. leprae* with other pathogenic mycobacteria, including *M. tuberculosis*, may provide insight into why *M. leprae* cannot be cultivated and how it is so successful in maintaining existence as a non-toxic intracellular parasite in some patients. Genomic information has already led to the identification of mutations associated with drug resistance and of genes controlling protein production that may be important immunologically. Identification of genomic patterns in strains of *M. leprae* may yield information that leads to a better understanding of its epidemiology and transmission.[6]

The standard diagnostic tests for *M. leprae* have been slit skin smears and punch biopsy of the skin. The former has low sensitivity and is difficult to maintain quality control. The latter is invasive and requires physicians to process and interpret the specimen. These resources are not available in all localities and thus skin biopsy will not be practical for broad use. The WHO field classification has proven to be useful but is likely to decrease in utility as leprosy becomes less common. It is also meant for less developed countries and does not have a major role in the United States. It seems likely that serologic diagnosis will not play a major role in leprosy control. While PCR has promise, the test will need to be modified to have better sensitivity and specificity, to be useful under field conditions, and to be less expensive.[194,195] Thus, improved methods for diagnosis and follow-up of patients are important and may become even more important as control programs are more and more integrated into primary health care. In addition, there are no reliable laboratory tests to diagnose leprosy immunologic reactions or to assess the severity of such reactions. Hopefully identification of the mechanisms underlying such reactions will lead to development of such diagnostic tests.

PCR has demonstrated the presence of *M. leprae* in the nose of individuals without clinical signs or symptoms of leprosy. Questions that must be answered include the following: (1) Is there a way to determine which of these individuals will develop clinical leprosy? (2) Are these individuals contagious during the period of nasal carriage? and (3) What is the typical duration of nasal carriage? Related to these questions is the question of the role of non-human sources of leprosy. Finally, PCR or some other test must be developed to aid in differentiating relapse from re-infection.

As discussed in Section 9.2, it is possible that 12 months of therapy is sufficient for most patients, and it is clear that some patients (PB and single lesion) can be treated with shorter courses of therapy. An important question is whether it is possible to select those MB patients who can be successfully treated with less than 24 or 12 months of therapy, just as it is clear that some patients (BI $\geq$ 4) require more than 24 months of therapy. Likewise, it is unclear whether newer

drugs, either those currently available or those yet to become available, will play a significant role in leprosy control. If the number of patients continues to fall, it will become increasingly difficult to conduct scientifically valid trials of new agents.

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CHAPTER 21

Leptospirosis

Paul N. Levett and Charles N. Edwards

1. Introduction

Leptospirosis in humans occurs globally[141] as an acute infection ranging in severity from unnoticed and subclinical to fatal. It is a zoonosis (strictly, an anthropozoonosis) acquired by humans from an animal source. The causal bacterium is a pathogenic spirochete of the genus *Leptospira*, of which there are several species and more than 250 serological varieties (serovars), each of which has some diagnostic, prognostic, or epidemiological significance.[50,70]

The ultimate reservoir of all leptospirosis is a non-human carrier animal that excretes the causal bacteria (leptospires) in its urine. In animals, the leptospires infect primarily young animals, which may become renal carriers and urinary excreters if they survive an acute initial infection. Human-to-human transmission has been recorded extremely rarely, congenital infection has been infrequently reported, and laboratory-acquired infections have been documented rather more frequently.

There are a large number of leptospires identified by serology or by genetic relationships. In general, there is a correlation between animal reservoir, serological type (serovar), and potential severity of the illness. The disease has many local and folk names, frequently originating from historical or clinical features or associated occupations, such as "pretibial fever," "swineherds' disease," "cane-cutters disease," "mud fever", "pea-pickers' disease" or "swamp fever".

As it is a zoonosis, leptospirosis is most significant for those whose work or leisure brings them into contact with infected animals or their urine. It is highly related to occupation in societies where animal contact is rare except through work. In tropical areas especially, it is impossible to avoid direct or indirect contact with feral carrier rodents or with livestock. In other areas, only those in selected occupations are exposed to significant risks. The public health importance of leptospirosis lies in its occupational, seasonal, and sex- and age-related incidence; the acute and sometimes prolonged incapacity for work; loss of human and livestock productivity; costs for medical care, workers' compensation, or similar insurance payments; and preventive measures. It can be epidemic, sporadic, or endemic.

2. Historical Background

The early history of leptospirosis is vague, because it was not possible to differentiate the different forms of "malignant jaundice," which included hepatitis, yellow fever, leptospirosis, and malaria, until scientific clinical and pathological knowledge had progressed into the 1880s. The history of leptospirosis was reviewed by Faine[50] and by Everard.[48] The clinical entity was described by both Mathieu and Weil in 1886, but Weil's description became the classical reference. The syndrome of fever, hemorrhage, jaundice, and enlarged liver and spleen, with renal failure, became known as "Weil's disease." Its etiology was unknown until the discovery of pathogenic leptospires, first seen in stained tissue sections of liver from a "yellow fever" patient, and later cultivated independently from Weil's disease patients among coal miners in Japan in 1914 and German troops in trenches during World War I in 1915. It was named *Spirochaeta icterohaemorrhagiae* by the Japanese; *Spirochaeta icterogenes* by the Germans; and later *Leptospira icterohaemorrhagiae* by Noguchi. Both the Japanese and German workers and their associates quickly published much of the basic knowledge of the microorganisms, as well as the pathogenesis, pathology, clinical features and diagnosis, and rodent source of leptospirosis.

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Less severe illnesses were soon recognized as forms of leptospirosis. Dogs were identified as both reservoirs and susceptible animals. Once the wider range of clinical manifestations was recognized, other serovars were isolated from patients and carrier rodents and were identified, so that by the mid-twentieth century, 45 serovars significant for human leptospirosis and their reservoirs had been identified.

A major discovery was that livestock (pigs, cattle) could be carriers and could excrete large numbers of leptospires in urine. Both primary (rodent–rodent) and secondary (rodent–livestock–rodent) cycles were identified. The range of known serovars continued to expand, mainly following research in tropical countries. Although leptospires could be isolated by culturing blood or urine from patients, evidence of leptospirosis was obtained mainly by serological tests on patients' sera. Most culture media required rabbit serum. The use of bovine albumin and oleic acid or polysorbate-linked fatty acids (Tween) to replace rabbit serum was an important advance, facilitating laboratory cultivation and allowing the easier isolation of serovar Hardjo, a major cause of human leptospirosis wherever there are dairy cattle. The subtype "hardjoprajitno" was originally isolated from an Indonesian patient, and the subtype "hardjobovis" later, from cattle. Antibodies to Hebdomadis-group leptospires were already known to be prevalent in both humans and cattle. Genetic typing of leptospires, first done in the 1960s by DNA–DNA hybridization, was a major step that led much later to formal recognition of genospecies of *Leptospira*.

Vaccines for control of leptospirosis in dogs were introduced in 1939 and in pigs and cattle soon after. These vaccines would also indirectly assist in protecting humans by reducing excretion of leptospires by animals. Further developments in knowledge of microbiology of leptospires and of pathology, pathogenesis, immunity, epidemiology, and control of leptospirosis are reflected in improved laboratory and diagnostic methods during the 1980s. Recent rapid progress in the understanding of the molecular biology of leptospires has led to the identification of several significant outer membrane antigens. The genomes of several strains have now been sequenced and comparative studies are underway.

3. Methodology

The diagnosis of leptospirosis usually depends on laboratory confirmatory tests. Laboratory tests may be unavailable or delayed, because the diagnosis is not considered at an early state or because the patient is not seen until late in the illness. Failure to use appropriate laboratory tests at the proper time can lead to misdiagnosis in both suspected and unsuspected patients. Because of diagnostic deficiencies, mortality, morbidity, and incidence data based on unconfirmed diagnoses or confirmed diagnoses of a few selected patients are fallacious, almost without exception.

3.1. Sources of Data

The World Health Organization (WHO) has recommended that leptospirosis be a notifiable disease. Notification may be based on hospital admissions and diagnosis, discharge data, notification by physicians, or laboratory notification of infections diagnosed by isolation of leptospires, or on seroconversion, or both. Laboratory notification results in the recording of higher morbidity rates than practitioner notification. This is partly because patients are discharged, or recover, or die before all laboratory results are returned to practitioners, who may then see notification as useless. Follow-up of patient contacts has led to detection of cases, not because of the contagiousness of the disease, but because local outbreaks have been detected as a result of inquiries.

Death notifications and autopsy reports have been used. Accurate postmortem diagnosis is impossible where autopsies are forbidden by custom or belief. Antemortem diagnosis is required for accurate death notification without an autopsy. Mortality rates may be spuriously high if the diagnosis is made only in advanced cases who are nearly moribund when medical or hospital assistance is sought.

Expression of mortality or morbidity statistics in terms of a total population can give a false index of incidence or prevalence if the disease is not homogeneously distributed in that population, or unless a correction is made for risk of exposure. In a large predominantly urban population, an overall low morbidity rate may mask a very high rate in a selected group of that population, such as sewer workers, abattoir workers, or meat packers. Statistics should be refined and expressed in rates relevant to the size of the population at risk.[50]

Leptospirosis is not a notifiable disease in many jurisdictions, including the USA, where the disease ceased to be nationally notifiable after December 1994[24] but remains so in some states.

3.2. Surveys

Serological surveys have been used frequently in studies of leptospirosis. They can be used on unselected populations to see whether leptospirosis exists at all. In such studies it is necessary to use a battery of antigens in agglutination tests to ensure that representative serovars of all serogroups are included. Locally prevalent serovars must be included, if they are known.

Survey information can be used to show which serovars are likely to be prevalent, by analyzing results of tests on batteries of antigens, or by retesting broadly reactive sera against individual serovars. Many sera will react with more than one serovar, however, because several serovars have antigens in common, and the highest titer is not necessarily against the infecting serovar, especially following recent infections. The fact that leptospirosis may not be distributed homogeneously in the population should be borne in mind when planning surveys. The survey should be properly planned to request necessary information such as age, sex, occupation, travel, and previous known leptospirosis or similar undiagnosed illness. Adequate statistical planning beforehand is essential to ensure that an appropriate number of samples are taken in each group and that results can be analyzed by statistical techniques.

Once the presence of leptospirosis is known or detected in a survey, the data may be used to determine the population groups exposed to a risk or risks and once those are delineated, to measure the prevalence of antibodies in one or more selected subgroups. Another important function of surveys is to ascertain prevalence for evaluating control measures, both for a baseline level before their introduction and during and after implementation as part of their evaluation.

The presence of IgM antibodies may be surveyed using ELISA, allowing large numbers of sera to be tested relatively quickly. IgM antibodies usually indicate relatively recent infections. Survey information can be important for interpretation of diagnostic serology in acute cases to establish whether the patient is one of a population or subgroup in which there is a known high prevalence of antibodies.

Serological or bacteriological surveys have proved valuable in investigating outbreaks. Information may be obtained retrospectively from serum bank specimens collected from epidemic patients or from patients and their surrounding population in general concurrently with an epidemic. Apart from epidemics, surveys of serum banks have been used to ascertain overall prevalence rates in certain clinical groups, such as patients with jaundice and fever, undiagnosed aseptic meningitis, or pyrexia of unknown origin.

Prospective surveys offer a better chance of understanding the incidence of leptospirosis and the prevalence of antibodies in selected groups. These may be carried out on selected groups of hospital admissions or patients in primary healthcare clinics or practices. A suitable protocol is to carry out diagnostic tests for leptospirosis on all clinically suspected patients.[41,45,46,88] If serology is tested, a matched control series, such as nonfebrile patients, must be included.

Collection of specimens for survey purposes requires prior ethical review and the obtaining of informed consent from individual survey subjects. Where surveys are carried out as part of national or international research programs, it is essential that local customs, traditions, and religious practices of the population are respected and the surveys are carried with their cooperation.[50]

3.3. Laboratory Diagnosis

In the absence of clear characteristic clinical features, especially in mild leptospirosis, laboratory diagnosis is essential. Its importance cannot be overstated, because it is also the basis of epidemiological statistics and preventive policies and programs. Not only must the diagnosis be established as leptospirosis, but the causal leptospire should be identified, at least by serogroup, and preferably also by isolation and identification of serovar and genospecies, for prognosis, management, and prevention activities.

3.3.1. Isolation and Identification of Leptospires

3.3.1.1. Classification and Nomenclature of Leptospires. All leptospires are similar in appearance and culture. Historically, they were classified into serovars and serogroups by serology using cross-agglutination with rabbit antisera and a microscopic endpoint.[37] All pathogens were grouped in a serologically defined species known for years as "*L. interrogans*," and nonpathogens were similarly grouped as "*L. biflexa*." Now the members of the genus *Leptospira* are classified by genetic relationships into distinct species (genospecies, see Table 1). Phylogenetic analysis shows the existence of three clades with the genus, comprised of pathogens, saprophytes and an intermediate group of uncertain pathogenicity (Figure 1). Genera of *Leptonema* and *Turneriella* are recognized, comprising morphologically similar spirochetes. Many strains are not yet classified by genotype. Several strains of leptospires classified serologically by their antigens as members of a single serovar are found to belong to different genospecies; that is, more than one species may express the same serovar-specific antigens.[51,64]

3.3.1.2. Culture. Culture media containing long-chain fatty acids such as oleic acid or Tween with 1% bovine serum albumin as a detoxicant are used widely. A common formulation is the Ellinghausen–McCullough–Johnson–Harris (EMJH) medium. It is usually used in liquid form in screw-cap containers. In tubes of semisolid media containing 0.1–0.2% agar, growth occurs in one or more zones (Dingers' rings) situated from just below the surface to deep in the medium. In fluid media, growth from inocula of 1–10% of volume can be seen as a birefringent swirl in 3–20 days, depending on the individual strain. Heavier growth, always less than the turbidity seen in cultures of enteric bacteria, may be encouraged by shaking during incubation. Optimum temperature for the cultivation of laboratory strains is 28–30°C. Most strains grow poorly if at all at 37°C.

Table 1. Species and Some Serovars and Serogroups of the Genus *Leptospira*

Species	Serovar	Serogroup	Serovar	Serogroup
Pathogens				
L. alexanderi	Lushui	Manhao	Nanding	Hebdomadis
L. borgpetersenii	Arborea	Ballum	Jules	Hebdomadis
	Balcanica	Sejroe	Mini	Mini
	Ballum	Ballum	Sejroe	Sejroe
	Hardjo (Hardjobovis)	Sejroe	Tarassovi	Tarassovi
	Javanica	Javanica		
L. interrogans	Australis	Australis	Icterohaemorrhagiae	Icterohaemorrhagiae
	Autumnalis	Autumnalis	Kennewicki	Pomona
	Bangkok	Australis	Kremastos	Pomona
	Bataviae	Bataviae	Lora	Australis
	Bratislava	Australis	Medanensis	Sejroe
	Broomi	Canicola	Mwogolo	Ictero.
	Bulgarica	Autumnalis	Naam	Ictero.
	Canicola	Canicola	Paidjan	Bataviae
	Copenhageni	Icterohaemorrhagiae	Pomona	Pomona
	Djasiman	Djasiman	Pyrogenes	Pyrogenes
	Hardjo (Hardjoprajitno)	Sejroe	Szwajizak	Mini
	Hebdomadis	Hebdomadis	Zanoni	Pyrogenes
L. kirschneri	Bim	Autumnalis	Mwogolo	Icterohaemorrhagiae
	Bulgarica	Autumnalis	Mozdok	Pomona
	Cynopteri	Cynopteri	Kunming	Pomona
	Grippotyphosa	Grippotyphosa		
L. noguchii	Bajan	Australis	Louisiana	Louisiana
	Fortbragg	Autumnalis	Panama	Panama
L. santarosai	Bataviae	Bataviae	Pyrogenes	Pyrogenes
	Borincana	Hebdomadis	Tabaquite	Mini
	Kremastos	Hebdomadis	Trinidad	Sejroe
	Navet	Tarassovi	Weaveri	Sarmin
L. weilii	Celledoni	Celledoni	Mengma	Javanica
	Hainan	Celledoni	Sarmin	Sarmin
Leptospira genomospecies 1	Sichuan	Undesignated	*Leptospira* genomospecies 1	Sichuan
Intermediate group				
L. broomii	undesignated	undesignated		
L. faineii	Hurstbridge	Hurstbridge		
L. inadai	Lyme	Lyme		
Nonpathogens				
L. biflexa	Patoc	Semaranga		
L. meyeri	Ranarum	Sejroe		
L. wolbachii	Codice	Codice		
Leptospira genomospecies 3	Holland	Undesignated		
Leptospira genomospecies 4	Hualin	Icterohaemorrhagiae		
Leptospira genomospecies 5	Saopaulo	Semaranga		

Cultures of properly collected clinical material cultured shortly after collection are highly sensitive and capable of detecting very small numbers of leptospires after incubation for from 2 or 3 days to several weeks. Cultures should be examined at least weekly and incubated for 4 weeks to 2–3 months.

3.3.1.3. Specimens for Culture. Blood cultures taken during the first few days (up to 10 days, especially in the first week) during the fever are frequently positive. Addition of 100 μg/ml of 5-fluorouracil is useful to reduce risks of overgrowth by bacteria from contaminated specimens. Special culture media are available for isolating fastidious leptospires currently found mainly in veterinary practice.[42]

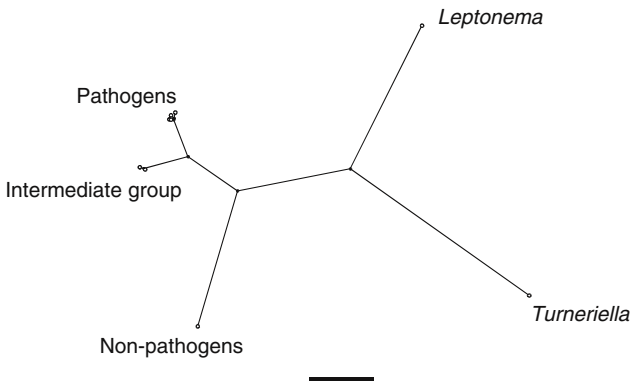


Figure 1. Phylogenetic relationships based on 16S rRNA gene sequences between the genera of the *Leptospiraceae*. The scale *bar* represents a 2% difference in sequence. See Table 1 for a list of species in the three clusters within *Leptospira*. Adapted from (72) with permission of the publisher.

Cultures may also be taken from cerebrospinal fluid (CSF), urine, and tissue samples, including autopsy specimens. Semisolid medium comprising EMJH with 0.1% agar and 100 μg/ml of 5-fluorouracil is useful for tissues.

3.3.1.4. Microscopy and Examination of Cultures. Dark-field microscopy by an experienced person is used to recognize leptospires in cultures. Leptospires appear as motile, bright, beaded, rotating, thin rods, usually with one or both ends curved, against a black background. Direct examination of clinical specimens by dark-field microscopy is both insensitive and nonspecific.[131]

3.3.1.5. Staining. Leptospires are poorly stained by conventional microscopic stains. They may be visualized by Giemsa or silver deposition methods. Silver deposition by one of several methods can be used for tissue sections, but flawless technique is required to obtain clean, interpretable results. Immunohistochemical staining has both greater sensitivity and specificity than other staining methods.[144] However, the major disadvantage of all immunostains is the need for specific antiserum. A battery of antisera may be required if the serovar is unknown or unsuspected. In extreme cases, where the infection is due to a new serovar, only convalescent patients' sera can be used as a source of the primary antibody for the test until the new organism has been isolated and immune sera prepared from it. Results of direct microscopy or immunostaining can be obtained in minutes to a few hours.

3.3.1.6. Serological Typing. If a leptospire is isolated, it must be identified by agglutination with reference antisera or serogroup- and serovar-specific monoclonal antibodies.[37,120] These identification procedures are usually performed only in reference laboratories. A list of species recognized currently, with the frequently isolated serovars and their serogroups, is provided in Table 1. A list of pathogenic serovars and their history[67] is now out of print, but is being revised.

3.3.1.7. Molecular Methods. A number of conventional PCR assays were developed for detection of leptospires, but few were subjected to clinical trials to evaluate their use in diagnosis of leptospirosis in man[18,87] or in animals.[61,135] The most widely used assays were based upon different approaches: one detected all pathogens using two quite different targets,[59] while the other used a genus-specific 16S rRNA target.[86]

Recently, real-time PCR assays have been developed, again using 16S rRNA targets.[89,116] However, recent advances in the understanding of the molecular biology of leptospires have led to the design of assays that target virulence factors expressed by pathogenic leptospires.[75,96] Real-time PCR assays are potentially more sensitive than conventional assays. One assay has been evaluated and shown to have sensitivity and specificity comparable to culture.[111]

The genospecies of *Leptospira* were defined by DNA–DNA hybridization,[16,99,102,143] but this is not a practical approach to identification of isolates. Sequence-based identification of *Leptospira* species offers an alternative, using 16S rRNA gene sequencing,[90] but within the nonpathogenic clade in particular the 16S rRNA sequences are highly conserved. Other targets may have better discriminatory power and are currently being investigated.[69,114]

Molecular methods employed for serovar identification have included digestion of chromosomal DNA by restriction endonucleases (REA), restriction fragment-length polymorphisms (RFLP), ribotyping, pulsed-field gel electrophoresis (PFGE), and a number of PCR-based approaches.[70] Currently, the most readily applicable molecular method for identification of serovars is PFGE.[62,63] This approach has been standardized using the PulseNet model.[55]

More recently, sequence-based methods such as amplified fragment-length polymorphism (AFLP) and multiple-locus variant repeat analysis (MLVA) methods have been developed for the study of leptospiral epidemiology.[79,105,112,113,130] The use of multi-locus sequence typing is being investigated.[2] The application of these tools will enhance understanding of leptospiral epidemiology at a population level.

3.3.2. Serological Tests. Antibodies in sera from patients or survey subjects can be detected and measured directly by agglutination of leptospires or by ELISA. Tests used for diagnoses of acutely ill patients need to be specific and performed rapidly if they are to aid in management and prognosis, as well as for detection of sources common to

several patients and for prevention. For epidemiological and survey purposes, there is less urgency for obtaining test results, but laboratories should be able to test large numbers of sera rapidly if necessary. Usually, knowledge of the serovar specificity of reacting antibodies is required, especially where several serovars are endemic, each with a different reservoir and pattern of transmission.

The microscopic agglutination test (MAT) is the gold standard test but requires significant expertise to maintain and interpret. IgM detection using ELISA is more widely used in diagnostic laboratories.

3.3.2.1. Agglutination Tests. The reference method for serological diagnosis of leptospirosis is the microscopic agglutination test (MAT), in which patients' sera are reacted with live antigen suspensions of leptospiral serovars. After incubation the serum/antigen mixtures are examined for agglutination by dark-field microscopy and the titers are determined. Appropriate quality control sera of known high- and low-specific reactivity are included as controls. The authenticity of strains used in antigen suspensions should be checked periodically against reference antisera. An external quality control program is organized by the International Leptospirosis Society.[26]

The range of antigens used should include serovars representative of all serogroups[125] and all locally common serovars. The highest titer is sometimes recorded against a cross-reacting serovar. Where several serovars from different serogroups are prevalent locally, there is a need to test each patient's serum against each of them or at least against a representative serovar of each serogroup. This means that in many tropical areas, or where the likely infecting serovar is unknown, a battery of up to 30 serovars may be used for each serum. There is no satisfactory single group antigen with which all patients' sera will react. The MAT is a serogroup-specific assay. Often, it becomes possible to distinguish a predominant serogroup only months after infection, as cross-reacting titers decline at different rates.[78] It is thus important if possible to examine serial convalescent specimens, in order to determine the presumptive infecting serogroup. The ability of convalescent MAT titers to predict the infecting serogroup may be as low as 40%.[71]

Interpretation of the MAT depends on the stage of illness, the serovar, and local epidemiology. Acute infection is suggested by a single elevated titre detected in association with an acute febrile illness. The magnitude of such a titre is dependent upon the background level of exposure in the population, and hence the seroprevalence. Thus a titre of $\geq$200 is used to define a probable case with a clinically compatible illness.[24] This may be appropriate for use in a population where exposure to leptospirosis is uncommon, but in most tropical countries a higher cut-off titer is necessary for defining probable cases of leptospirosis. In endemic areas a single titer of $\geq$800 in symptomatic patients is generally indicative of leptospirosis.

Paired sera are required to confirm a diagnosis with certainty. A fourfold or greater rise in titre between paired sera confirms the diagnosis, regardless of the interval between samples. The interval between first and second samples depends on the delay between onset of symptoms and presentation of the patient. If symptoms typical of leptospirosis are present then an interval of 3–5 days may be adequate to detect rising titers. However, if the patient presents earlier in the course of the disease, or if the date of onset is not known precisely, then an interval of 10–14 days between samples is more appropriate. Less often, seroconversion does not occur with such rapidity, and a longer interval between samples (or repeated sampling) is necessary. MAT serology is insensitive, particularly in early acute-phase specimens.[32]

MAT interpretation is complicated by cross-reactivity that occurs between different serogroups, especially in acute-phase samples. Titers following acute infection may be extremely high ($\geq$25,600) and may take months, or even years, to fall to low levels.[33,78,104] Rarely, seroconversion may be delayed for many weeks after recovery, and longer serological follow-up will be necessary to confirm the diagnosis.

The MAT is the most appropriate test to employ in epidemiological sero-surveys, since it can be applied to sera from any animal species, and because the range of antigens utilized can be expanded or decreased as required. Only one specimen is required for epidemiological and prevalence surveys. Serum may be frozen during transport and storage. It is usual to use a titer $\geq$100 as evidence of past exposure.[50] However, conclusions about infecting serovars cannot be drawn without isolates; at best the MAT data can give a general impression about which serogroups are present within a population.[71] Antibodies persist following clinical or subclinical infection for long periods.[11,33,78]

The MAT is a complex test to control, perform, and interpret.[125] Live cultures must be maintained of all the serovars required for use as antigens. This applies equally whether the test is performed with live or formalin-killed antigens. The labor-intensive nature of the test leads to observer error and fatigue following prolonged reading of tests. Interpretive errors may result from the use of inappropriate serovars in the panel of antigens. Direct measurement of specific IgM or IgG in MAT is not feasible. The repeated weekly subculture of large numbers of strains presents hazards for laboratory workers and laboratory-acquired infections have been reported.[4,100] Other drawbacks include the continuous risk of cross-contamination of the antigen cul-

tures, necessitating periodic verification of the identity of each serovar. MAT titers are also affected by the culture medium in which the antigens are grown.

3.3.2.2. Enzyme Immunoassay (ELISA). Detection of IgM antibodies by ELISA has been widely applied[1,121] for diagnosis of human leptospirosis. ELISA products are available commercially in several formats[74,115,140] and are widely used for screening specimens before testing by MAT. ELISA tests do not measure the same antibodies as those in agglutination tests, so there is little correspondence between the optical densities or titers in ELISA and the agglutinating titer in MAT. In pigs and cattle, blood levels of IgM reacting in ELISA have been correlated with renal excretion of leptospires.

4. Biological Characteristics of the Organism

Leptospires are tightly coiled spirochaetes, usually 0.1 μm × 6–20 μm, but occasional cultures may contain much longer cells. The helical amplitude is approximately 0.1–0.15 μm and the wavelength approximately 0.5 μm.[50] The cells have pointed ends, either or both of which are usually bent into a distinctive hook (Figure 2). Two axial filaments (periplasmic flagella), with polar insertions, are located in the periplasmic space.[118] Leptospires exhibit two distinct forms of movement, either translational or rotational. Dark-field microscopy of a wet preparation shows leptospires rotating rapidly around their long axis and translationally motile in either direction. One or both ends are usually hooked, imparting a looped appearance to the end of the leptospire when it rotates rapidly. In semisolid agar media, movement is slowed. Morphologically all leptospires are indistinguishable, but morphology of individual isolates varies with subculture in vitro and can be restored by passage in hamsters. Leptospiral lipopolysaccharide has a similar composition to that of other gram-negative bacteria.[20]

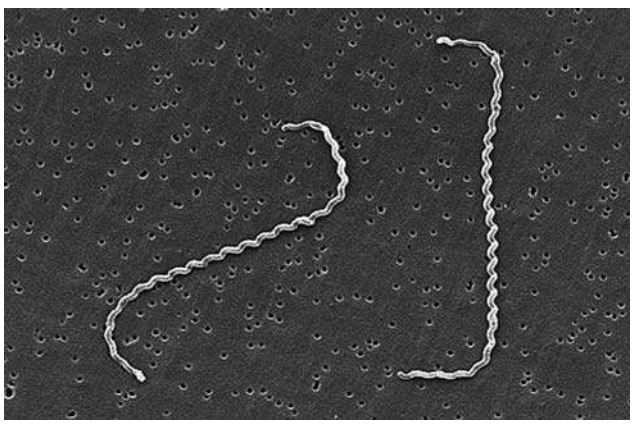


Figure 2. Scanning electron micrograph of leptospiral cells bound to a 0.2 μm filter. Magnification, approximately ×3,500. (Courtesy of Janice Carr, Public Health Image Library, Centers for Disease Control and Prevention).

All members of the genus *Leptospira* are obligate aerobes or microaerophiles with an optimum growth temperature of 28–30°C at pH from 7.2 to 7.8. They produce catalase, oxidase, and urease. Many strains produce lipase. They grow in simple media enriched with vitamins (vitamins B2 and B12 are growth factors), long-chain fatty acids and ammonium salts. Long-chain fatty acids are utilized as the sole carbon source and are metabolized by β-oxidation.[64] Phenotypic tests are not helpful for identification of leptospires, which must be identified by a combination of molecular and serological approaches.

Leptospires are easily killed by drying, by moderate heat (above 42–45°C for a few minutes), by acid conditions (below pH 7.0) or alkaline conditions (above approximately pH 7.8), and by disinfectants such as phenolics, quaternary ammonium derivatives, halogens, and aldehydes (formaldehyde, glutaraldehyde).

The leptospiral genome is comprised of two chromosomes, approximately 3500–4300 kb and 300–350 kb in length respectively. The genome of *L. interrogans* is approximately 4800 kb in size,[95,103] while that of *L. borgpetersenii* is approximately 15% smaller.[21] Leptospires contain variable numbers of rRNA genes, which are widely spaced on the larger chromosome. Numerous repetitive elements have been identified; several are insertion sequences, and are found in many serovars, but the copy number varies widely between different serovars and among isolates of the same serovar.[14] Genomic rearrangements resulting from multiple translocations have been identified.[21,95]

A wide range of antibiotics is effective against leptospires in the laboratory, although penicillin, cefotaxime, ceftriaxone, and doxycycline are widely used therapeutic agents, and doxycycline is recommended for chemoprophylaxis.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Statistics of prevalence and incidence are influenced frequently by the lack of reliability in diagnosis and by failure to recognize the selective risk to population groups. Prevalence figures derived from planned surveys are much more reliable than those based on hospital records or disease notifications. Antibody prevalence rates in tropical countries with

large feral and peridomiciliary rodent populations are much higher than in temperate climates, where leptospirosis tends to be more occupationally segregated.

Reported statistics are also influenced by technical factors such as the starting dilution for sera, the interpretation of low titers, and the techniques used for serology. These factors are often not clear from reports. When using prevalence statistics for epidemiological and control policy planning, it should be noted that the duration of persistence of antibodies after infection is variable.

Second infections with a different serovar have been reported, indicating that there is little cross-immunity between serogroups. Very rare reports of second infections with the same serovar are poorly documented.

In the United States, approximately 100 cases of leptospirosis are reported each year; however, the disease is not notifiable in many states and national data have not been collected for over 10 years. Cases occur throughout the country, but they are most common in tropical regions (Hawaii and Puerto Rico).

5.2. Epidemic Behavior and Contagiousness

Leptospirosis is not considered to be contagious, because spread from human to human is almost unknown and direct inoculation into the body via skin, mucosal, or conjunctival penetration is required for infection. Laboratory infections, a significant occupational problem, have occurred after inoculation into the eye or through the skin.[100] In natural infections an animal source of infection is required, with the rare exception of transplacental infection. Animal urine is the most important vehicle of infection for man, but direct contact with infected animals, their tissues or other body fluids are also important methods of transmission.

Significant changes in the epidemiology of leptospirosis have occurred in recent years. In developed countries the disease is much less prevalent than it was in the mid-twentieth century, for several reasons. Increasing urbanization has reduced the opportunity for exposure in the rural environment and immunization has largely controlled the disease in domestic animals. However, against this background there remain foci of infection, such as the recognition of continuing rat-associated leptospirosis in inner cities[132] and the emergence of canine infection in Eastern North America caused by serovars Grippotyphosa and Pomona,[101,136,137] presumably acquired from racoons, skunks or possums. There has been a significant rise in travel-associated leptospirosis, often associated with adventure tourism in the tropics,[25,109] and invariably associated with exposure to fresh water. By far the greatest epidemiological change globally has been the recognition of large outbreaks of leptospirosis associated with excess rainfall.[68,91,122,142] These outbreaks often occur in regions where diagnostic capacity is least well developed.

Although leptospirosis is a zoonosis, human social factors are most important in epidemiology. Occupational exposure to animals and contaminated environments is important, but avocational factors, such as housing conditions, sanitation, food storage conditions, livestock and domestic animals, and social and religious customs all affect the likelihood of exposure to leptospirosis.

5.3. Geographical Distribution

Leptospirosis is distributed worldwide,[141] wherever fresh water and carrier animals occur. There is a direct relationship with soil and geologic environment, which can affect the ability of leptospires to survive in surface waters and mud and soil through their effects on acidity and porosity.[65] Environmental changes will affect the magnitude of the risk of human leptospirosis, either positively or negatively.[98,133]

5.4. Temporal Distribution

Throughout the world, leptospirosis is related to wet periods of the year. The wet seasons, whether monsoon, floods, or spring and summer, are associated with surface conditions favoring leptospirosis in all parts of the world. In temperate climates the risks increase with fresh spring and summer growth of pastures. Risks also increase for harvest workers for many crops but not wheat or corn. Well-documented epidemics have been recorded during rice, sugarcane, and pea harvests.

5.5. Age

Although leptospiral antibodies can be found in people of all ages, the predominant incidence of acute infection is found in the 20- to 50-year age range, though in developing countries more cases may be seen in younger people. Pediatric and congenital infections have been described. The age distribution reflects the relative amount of environmental exposure to risk factors in the age groups concerned

5.6. Sex

Leptospirosis is predominantly a disease of males between the ages of 10 and 59 years. The case-fatality rate is age-dependent, rising to almost 30% in elderly adults.

5.7. Race

There is no evidence for any risk factors determined by race. Very little attention has been paid to genetic determinants of susceptibility to leptospirosis, but one recent study has shown that HLA-DQ6-positive triathletes were at greater risk of acquiring leptospirosis.[76]

5.8. Occupation

There is a strong occupational trend in all regions. Even in tropical climates, those whose jobs bring them into indirect contact with animals via rodent urine contamination in such activities as rice planting and harvesting, fish farming, forestry, mining, boating, and military exercises are at special risk. In all climates those exposed to urine contamination directly or indirectly from livestock and domestic dogs and rodents have a greater risk and increased incidence of infection. Occupational hazards involve special considerations of preventive measures to protect workers. Some countries have workers' compensation schemes which apply to persons who acquire leptospirosis at work.

5.9. Occurrence in Different Settings

Leptospirosis poses a special problem after natural disasters or in case of civil disruption. Rodent infestation may increase and with it risks to humans from leptospirosis as well as other zoonoses. Military, paramilitary, police, or civilian emergency workers may be forced into conditions such as floods and destroyed buildings that house rodents. Frequently, troops have been exposed to leptospirosis in rat-infested trench warfare or in jungle warfare or exercises. Modern leisure activities have attracted city dwellers to rural settings, sometimes in faraway lands, in search of adventures in canoeing, rafting, caving, and climbing. The risks of contracting leptospirosis on these vacations need to be emphasized, but it is important to recognize that the activities themselves put the participants at risk in temperate regions as well as tropical regions.[12,84,91,110,124]

5.10. Socioeconomic Factors

It is difficult to calculate the costs to society of the effects of leptospirosis. Some of the factors to be considered are the costs of labor to replace sick workers; the cost to the self-employed or peasant farmer or villager in money to replace his own inability to produce food for his family, and in pain and sometimes chronic illness; costs of medical attention including hospital and medical services; and costs in insurance for lost work, production, and workers' compensation. The first requirement is adequate diagnosis so that the actual impact of the disease can be assessed. However, costs of special diagnostic services and surveillance for leptospirosis are hard to justify if there is an impression that the disease is rare or absent, so that the circular logic leads to continued underdiagnosis and underreporting. Existing mild illnesses have frequently and easily been attributed to numerous other causes, including "influenza" or "viral infections", but may well be leptospirosis. Moreover, when diagnostic capacity does exist, the erroneous perception may occur that leptospirosis is more prevalent than in neighboring regions where no cases are diagnosed.[49]

An important socioeconomic consideration is the effect of leptospirosis on productivity of animals used for food or burden. Leptospirosis can cause abortions, stillbirths, failure to thrive, retarded growth, milk failure or spoilage for human consumption, and loss of productivity in meat animals among livestock. These effects can in themselves place an additional burden of malnutrition on people already threatened by the risk of illness.

6. Mechanisms and Routes of Transmission

Leptospirosis is transmitted primarily from its animal host reservoirs by urine. Excretion in urine may be intermittent or continuous and may contain very low numbers or very large numbers of leptospires; all of the former may occur at different times in a single animal. The reasons for the variable output are not known. Urine from carriers frequently contains antibodies to the homologous serovar of leptospires. The intermittent shedding, the difficulties of detecting small numbers of urinary leptospires in livestock, and the potentially enormous load of leptospires excreted into the surface water and soil environment from heavy feral rodent environmental contamination are the main sources of difficulties in control in different situations.

Pathogenic leptospires can survive free in the environment, depending on soil type and geologic factors in moist conditions such as in soil, mud, swamps, drains, surface waters, streams, and rivers, as long as conditions are not acid.[58] All of these sites are well known as sources of leptospirosis.[5,36] The leptospires can infect fresh host animals or humans via broken skin or mucosal surfaces.

Food preparation areas can be contaminated by infectious urine from foraging carrier rodents, resulting in infections in food process workers. Urine splash and aerosols can infect milkers (especially in "herringbone"-pattern milking sheds), veterinarians, and animal handlers. Animal tissues and blood are infrequently causes of leptospirosis, more often so in workers, inspectors, and transporters of meat. Conception products and autopsy of infected livestock and

separated kidneys used for domestic food can transmit leptospirosis to veterinarians, farmers, laboratory workers, and food-handlers and cooks.

Numerous outbreaks associated with water have been reported.[70] Spread of leptospirosis is not known to occur by airborne, respiratory, or gastrointestinal routes. However, immersion in, or ingestion of, water is a risk factor in other outbreaks[22,91] and it seems probable that leptospires can penetrate the oral or nasal mucosa. Human-to-human transmission is virtually unknown, apart from in utero congenital infection.

Laboratory-acquired infections usually have resulted from accidents involving direct skin penetration with cultures or suspensions of infected tissues via syringe and needle, broken glass, or splash into an unprotected eye, and from being bitten by infected animals (usually rats) whose urine contaminated the bite.

Leptospires die rapidly when dry. Drying out of surfaces or environments contaminated by infective material prevents spread, even after rehydration. Heating above about 42°C is lethal to leptospires, but they survive freezing.

7. Pathogenesis and Immunity

7.1. Incubation Period

The incubation period of leptospirosis varies from 2 to 21 days, usually between 3 and 10 days, although longer periods have been reported. It is relatively shorter with larger infective doses or more virulent leptospires. Incubation periods can be calculated from a recognized infection episode, but they are difficult to define when there is continual or repeated exposure. The relatively sudden onset makes it easier to detect the first clinical symptoms unless they are very mild or unnoticed. In these cases the incubation period may be erroneously dated to the onset of late presenting symptoms such as hemorrhage, meningitis, or renal failure.

7.2. Virulence and Its Attributes

The mechanisms by which leptospires cause disease are not well understood. A number of putative virulence factors have been demonstrated, but with few exceptions their role in pathogenesis remains unclear. Toxin production and adhesion have been shown by some strains in vitro and in vivo. Surface antigens clearly play an important role in virulence. The lipopolysaccharide (LPS) of leptospires is only weakly endotoxic. Early studies demonstrated the importance of LPS as a target of the immune response,[50] and recent data suggest that the O antigen content is modulated in acute versus chronic infections.[93] Much recent work has focused on the role of surface lipoproteins as potential virulence factors.[31] Many of the surface exposed lipoproteins such as OMPL1, LipL21, LipL32, LipL41, LipL45, and LipL46 are up-regulated in vivo, while others such as LipL36 are down-regulated.[82,94] LigA and LigB are adhesins, the expression of which is induced by physiological osmolarity.[27,81] Other adhesins have also been described recently.[9]

Genomic analysis has shown the existence of several hemolysins in *L. interrogans*.[145] Lk73.5 is a host-inducible sphingomyelinase in *L. interrogans* that is not produced in vitro.[7] LipL32, the predominant surface protein, is a hemolysin.[15] LruA and LruB are inner membrane lipoproteins that share epitopes with equine corneal proteins.[127] IgG and IgA antibodies to these proteins are found in uveitic eye fluids. Other proteins have been shown to bind factor H and this contributes to serum resistance.[85,128]

7.3. The Course of Events in Infection

7.3.1. Entry. Leptospires gain entry through small abrasions or cuts in the skin or mucosal surfaces. Entry may occur via the conjunctiva or by aerosol into the lungs, as well as by inhalation following immersion in contaminated water. As described above, ingestion of water probably results in penetration of the oral or nasal mucosa. Laboratory infections have occurred through injections, needle-stick injuries and cuts, and eyesplash accidents.

7.3.2. Spread. Leptospires that enter the body spread at once in the lymphatics and bloodstream. There is no initial localizing acute inflammation at the site of entry. Leptospires can be found distributed in various tissues within 2 h of infection. Both TLR-2 and TLR-4-dependent mechanisms appear to be important in the activation of the innate immune system.[134,139] Nonpathogenic leptospires and avirulent strains of the pathogenic species of *Leptospira* are opsonized by innate serum IgM. All leptospires may be opsonized by specific immune IgM or IgG in hosts previously immunized by infection or vaccination. Phagocytosis occurs rapidly in reticuloendothelial fixed phagocytes in the liver (Kupffer cells) and lung. These phagocytes later migrate to the spleen. The bloodstream is cleared rapidly following opsonization, which may occur with minimal detectable levels of immunoglobulin recognized as agglutinating antibody. Polymorphonuclear and mononuclear phagocytes can be observed to take up leptospires from the tissues following the development of antibody.

In the absence of immunity and consequent phagocytosis, leptospires grow in the body in an initial infection as if in culture. Nothing is known of specific nutritional requirements in vivo. Both virulent and avirulent leptospires

have been shown to adhere to fibroblast-derived cells in the laboratory.[8]

A threshold level of leptospires is required in the blood before lesions occur. In severe leptospirosis approximately 10^4 leptospires per ml blood are associated with a poor outcome.[107,123] The first and most important lesions to be detected are in small blood vessels throughout the body. Localized endothelial cell degeneration occurs, followed by small localized extravasations that frequently contain leptospires.

Fever occurs at an early stage of infection. At this stage it is also possible to demonstrate microscopic focal degeneration of skeletal muscle fibers, notably in the calf muscles. The extreme pain in muscles and acute tenderness found clinically are presumably the result of these lesions. Thus, the main presenting symptoms of severe headache, fever, and muscle pains can be attributed to the effects of leptospires growing in the body to threshold levels sufficient to cause characteristic damage to small blood vessels.

The natural history of the further development of leptospirosis in either humans or animals is similar. Exponentially increasing numbers of leptospires lead to severe blood vessel damage, local ischemia, and small hemorrhages in most tissues, and eventually to major organ damage. Depending on the serovar of leptospire, there is greater or lesser hepatocellular degeneration, leading to jaundice; renal tubular degeneration similar to that seen in crush injuries or after renal ischemias, leading to an acute, often hemorrhagic interstitial nephritis; and pulmonary hemorrhages ranging from insignificant to gross and fatal, with hemoptysis. Cholecystitis, symptoms of acute abdominal emergency, meningitis, hemiparesis, myocarditis, adrenal hemorrhage, and placentitis have all been reported.

7.3.3. Lesions. Once the infection is fully developed, the typical lesions described in clinical–pathological and postmortem studies of leptospirosis, usually of the severe and fatal types, appear. Hemorrhage is associated with a sudden drop in platelet numbers, possibly as a result of leptospiral toxin action or of adhesion of leptospires. The potential role of disseminated intravascular coagulopathy syndrome is not clear.[39] Anemia is common, usually not as a result of hemorrhage alone. Evidence of nitrogen retention is found almost universally, albeit transitorily, reflecting the renal ischemia occurring even in mild types of leptospirosis. In these cases, patients whose kidneys are functioning poorly and at the limit of compensation due to other causes of renal insufficiency may be precipitated rapidly into renal failure. In severe cases there is very widespread tubular degeneration, which is the usual main cause of death. Other potentially fatal lesions include hepatocellular degeneration, pulmonary hemorrhages, and myocarditis.[6,34,35]

7.3.4. Transplacental Spread. Transplacental infection can occur at any stage of leptospirosis. If the fetus or placenta is severely damaged, abortion will occur. Stillbirth or congenital leptospirosis may follow infection late in pregnancy. Abortion is a well-documented cause of loss of productivity of livestock. In human congenital infection, IgM antibodies can be demonstrated in the fetus and cord blood and leptospires in the degenerative lesions in tissues.[50]

7.3.5. Recovery. Recovery from leptospirosis commences as soon as the patient produces opsonizing antibody, which leads to rapid phagocytosis of leptospires in the circulation and in the tissues. Mopping up phagocytosis also follows destruction of leptospires by antibiotics. Opsonizing antibody levels can be correlated with agglutinating antibody levels. Virtually any detectable specific agglutinating antibody in MAT can signify opsonic activity. Bacteriologic clearance of live leptospires from tissues by antibody, however, does not ensure clinical recovery, especially in severe leptospirosis, because lesions in organs and tissue damage may have proceeded to stages where vital functions are almost irreversibly impaired. Clinical supportive measures for renal failure (dialysis), hemorrhage (transfusion), myocarditis, and liver failure may be necessary until the patient's tissues regenerate. Complete recovery is the rule in surviving patients, although rare examples of prolonged asymptomatic renal carriage have been recorded in people.

7.3.6. Carrier State. The carrier state occurs very rarely, if at all, in humans. This is assumed to result from the acidic reaction of human urine. In animals it follows recovery from systemic infection, which may be subclinical. Leptospires are attached to the epithelial surface of proximal renal tubule cells, where they grow in the tubular lumen and are excreted in the urine. Homologous antibody may be demonstrated in the urine of carriers. Active excretion may be sporadic or intermittent. The mechanisms of adhesion, immunologic events, and nutritional needs of leptospires in renal tubules need to be elucidated. There is no inflammation around affected tubules, which appear to be morphologically intact in electron microscopic studies. In some animals (especially dogs and pigs) there may be considerable scarring in the kidneys where leptospires occupy renal tubules, apparently related more to the recovery and repair processes following renal damage (acute nephritis) and hemorrhage in the acute state of infection than to the presence of leptospires in the tubules. Leptospires are not found in renal tissue outside renal tubules in carrier animals. Genital carriage of leptospires has been described in livestock.

7.3.7. Autoimmunity and Hypersensitivity. The roles of autoimmunity and hypersensitivity in producing lesions are unclear. The acute nature of infection and absence of chronic infection precludes consideration of

these immunopathologic mechanisms in the development of initial lesions. However, leptospires may be sequestered in "privileged sites" during acute infection, leading to later allergic reactions to their presence. A notable example is uveitis that has followed either systemic infection or accidental laboratory inoculation into the conjunctiva. Leptospires may be cultured from the anterior chamber of the eye for long periods after infection. Contact with leptospiral antigen can cause an allergic aggravation of the uveitis. A similar condition known as equine recurrent uveitis or "moon blindness" occurs in horses and results from antigenic mimicry between epitopes on equine cornea and an antigen expressed by some *Leptospira* serovars associated with equine uveitis.[77]

7.4. Immunity

7.4.1. Specific and Nonspecific Immunity. Low levels of innate antileptospiral IgM antibodies, found in the serum of humans and other animals not known to have been exposed to leptospirosis, destroy avirulent leptospires but are inactive against virulent strains. Nothing is known of nonspecific immunity to leptospirosis stimulated by infection with other bacteria or their LPS.

Specific immunity to leptospirosis develops rapidly, within a few days of clinical or subclinical infection. Fully effective immunity can be transmitted passively naturally through the placenta and colostrum and artificially with convalescent or hyperimmune serum. Both IgM and IgG antibodies are protective, and presumably colostral IgA antibodies are also, although there is no direct evidence. Immunity is associated with agglutinating antibodies which are also opsonic; moreover, a very low level of agglutinating antibody is protective. Immunity to reinfection is specific for the infecting serovar or closely antigenically related serovars, almost exclusively within the same serogroup determined by MAT.

Some animal species are relatively resistant to leptospiral infection with certain serovars; the basis of such resistance is unknown. It is not known whether humans are incapable of infection with any serovars. Generally, young animals are much more susceptible than old, provided they have no maternally endowed passive immunity. The development of age-related relative resistance to leptospirosis reflects the rate of maturation of the B-cell immune response.

The humoral immune response induced by immunization protects against disease but not against infection and subsequent renal shedding. Cell-mediated responses occur following infection and following immunization with newer vaccines[92]; there appears to be a lower degree of cell-mediated immunity following natural infection. In contrast to the traditional vaccines, cattle immunized with these vaccines are protected against renal colonization and urinary shedding.[13] Recent evidence suggests that a Th1 immune response also occurs in humans.[66,129]

The duration of immunity in humans is not known. Agglutinating antibodies fall in titer after infection, but the rate is very variable in individuals and there are no studies of the relative rates of fall of IgM and IgG antibodies. Some people known to have had leptospirosis are seronegative in 1 or 2 years and others are still seropositive after more than 10 years, although the possibility cannot be excluded that they have had continued exposure to leptospires during this period.[33,44] It is not clear that the loss of detectable agglutinating antibody over time following infection is synonymous with loss of immunity. These deficiencies in knowledge are obviously important for understanding immunity, epidemiology, and planning for vaccines.

7.4.2. Antibodies. Convalescent sera contain specific IgM, IgG, or both. Usually, IgM is elaborated first, within approximately 7–20 days of infection, followed by IgG. In some patients, seroconversion does not occur until 30 days or more after onset of infection. IgM persists at low levels in certain cases, while in others IgG is sometimes the sole demonstrable immunoglobulin. However, information from this type of retrospective study is necessarily limited in value because one cannot know for certain beforehand the previous history of exposure to leptospirosis. Cross-reactions with related and occasionally with unrelated serovars occur, especially early in infection. These "paradoxical reactions" occur typically, for example, between serovars Icterohaemorrhagiae and Canicola, and Icterohaemorrhagiae and Pomona. IgG antibodies tend to be more specific. In some patients treated very early with penicillin whose infections were proved with blood cultures, seroconversion did not occur at all or was delayed several weeks. Antibodies to the infecting serovar may be found in the urine of carrier animals.[50]

7.4.3. Antigens Related to Pathogenesis and Immunity. Antibodies reacting with antigens in flagellar, subsurface, and outer-envelope locations have been identified in sera from patients who have been proved to have leptospirosis. Some of the antibodies persist for months and years.[11,33,78]

7.4.4. Vaccines. Vaccines for leptospirosis were introduced soon after leptospires were identified and could be cultivated. Preparations effective in protecting animals and humans have been made by killing the leptospires in cultures with heat or formalin and injecting doses subcutaneously or intramuscularly. Formalin-killed cultures are used widely to vaccinate animals (cattle, pigs, dogs) and are in use in some countries to vaccinate people. The vaccine is usually given subcutaneously in two doses and repeated

annually. There is a high incidence of painful swellings at the injection site, especially on revaccination.

In countries where leptospirosis is confined to clearly recognized occupational groups whose source of infection is livestock, the vaccination of livestock is recommended and used widely to decrease shedding by carrier animals with the twofold purpose of reducing and eliminating animal leptospirosis, thus increasing productivity, and protecting humans at risk from unavoidable exposure to these animals. Pregnant sows are commonly vaccinated to ensure passive protection to growing piglets. However, piglets may acquire asymptomatic leptospirosis after their passive protection has been lost, and they may then become shedders and thus a hazard to handlers, slaughtermen, and other abattoir workers.[23] Similarly, cattle that have been vaccinated are protected against disease but not against infection. The major limitation to use of vaccines in dogs is the potential for infection with serovars that are not included in the vaccine. Vaccinated dogs may thus represent a source of human infection.[47,53]

More recently a new generation of vaccines have been introduced that induce cell-mediated immunity in cattle.[19,92] Moreover, immunization with such vaccines prevents both disease and urinary shedding.[13]

8. Patterns of Host Response

Several epidemiological features of leptospirosis determine the host responses and clinical features. The disease is almost uniquely acquired from carrier animals, which cannot be identified as carriers or shedders without laboratory tests. Inapparent infections in humans are not relevant to clinical disease or transmission.

The severity and presenting features of clinical leptospirosis result from a complex of climatic, social, and geographic factors influencing the animal host and source, and thus the serovar and the clinical features and in turn the accuracy of diagnosis and notification. In general, different leptospiral serovars are associated with characteristic groups of animal species.

Living conditions for most people in tropical countries favor contact with rodent sources of leptospires rather than other animals. Most of the leptospires that clinically result in the very severe leptospirosis of the classical Weil's disease type, which may be fatal following renal failure, hemorrhages, and jaundice, are carried by rats. Serovars of the Icterohaemorrhagiae, Autumnalis, and Bataviae serogroups are examples.

Conversely, in temperate climates, in addition to rats, there are other carriers of leptospires such as field mice, pigs, dogs, and cattle that shed mainly leptospires of those serovars causing a milder form of illness. The disease also tends to be much more occupationally related and confined to people in contact with these animal sources. Serovars Grippotyphosa, Hardjo, Pomona, and Tarassovi are examples of this group.

Perceptions and statistics of leptospirosis are influenced by whether the disease is seen as the severe or the mild type. Mild infections of either variety have often been overlooked because the symptoms are not pathognomonic. Usually, seriously ill patients who needed hospital treatment or died were accurately diagnosed as leptospirosis. Since they were the only ones in whom the diagnosis was made, the clinical features recorded and reported in textbooks have been those of the severe type, emphasizing jaundice, hemorrhages, renal failure, meningitis, and a high case-fatality rate. Overall, these symptoms appear in only a few and late cases of the classical syndrome of Weil's disease. Most patients affected with any serovar report initial symptoms as described below. The severe and the mild types differ in their subsequent progress.

8.1. Clinical Features

The clinical features are usually divided into the mild anicteric phase,[10] accounting for about 90% of all cases, and the severe or icteric disease causing the syndrome Weil's disease.[41] Characteristically, leptospirosis commences suddenly with headache, myalgia, fever, and red eyes. Headache develops without warning and may be extremely severe. Myalgia is usually felt in the back and especially in the calf muscles, which may be excruciatingly tender to touch. The fever is generally greater than 39°C and the red eyes are the result of conjunctival suffusion, reflecting a generalized dilation of blood vessels, rather than a conjunctivitis. There is frequently a macular rash on the trunk, lasting only a few hours. Careful examination of the palate may show a similar petechial rash. In untreated patients with the mild type of leptospirosis, the symptoms usually persist for 3–10 days, followed by gradual but complete recovery. Occasionally, further acute symptoms may develop. These include oliguria from renal failure; acute abdominal pain, resembling an acute abdominal emergency and sometimes due to acute cholecystitis or pancreatitis; meningism, hemiparesis, or other neurological signs; and cough, sometimes with mild hemoptysis. Jaundice is rare in mild-type leptospirosis. Aseptic meningitis may develop as a later complication, but most patients recover fully within 3–6 weeks.

In patients with severe leptospirosis, the symptoms increase in severity, at times following a 1- or 2-day temporary remission. Delirium and confusion may develop, with increasing evidence of renal failure, together with hemorrhages and jaundice. In addition, the spleen may be enlarged.

Pulmonary hemorrhages may be diffuse, resembling multiple infarcts or bronchopneumonia, accompanied by coughing of blood, but in some cases the pulmonary hemorrhages and hemoptysis may be gross. Adult respiratory distress syndrome can also occur and cause death. As liver and renal failure progress, jaundice and oliguria increase, confusion and delirium give way to unconsciousness, and death occurs through renal and hepatic failure. Supportive measures such as dialysis can tide patients over renal failure until renal structure is regenerated and function restored. Myocarditis, evidenced by arrhythmias, can cause sudden death. Final recovery is usually complete unless vital organs are irreparably damaged. The earlier severe phase is described as the septicemic stage and the later the tissue phase.

Death from renal failure may occur at any state of the illness. The autopsy appearances will depend on the duration and degree of development of lesions. Usually, there is generalized jaundice and widespread hemorrhage in the skin, muscles, peritoneal surfaces, and lungs. The kidneys are enlarged, bile stained, and have subcapsular hemorrhages. Bleeding into all viscera including the adrenals is common. The liver shows signs of hepatitis and hemorrhages. Death is so rare in mild cases that there is no record of significant and characteristic autopsy appearances. Uveitis may develop at any time after the second week, and as late as 6 months. It may affect one or both eyes.[80]

8.1.1. Congenital Leptospirosis. Leptospirosis in pregnant women can be transmitted to the developing fetus at any state of pregnancy.[28,52] The fetus is actively infected. If it is severely damaged, even by serovars causing the mild type of leptospirosis in the mother, it may die.[3,29] If infection occurs near the time of birth, the child may develop congenital neonatal leptospirosis, whose manifestations are similar to those of adult leptospirosis. Complete recovery follows satisfactory treatment. The diagnosis of intrauterine leptospirosis depends on the correct diagnosis in the mother and evidence that the fetus is infected as shown by fetal distress. Antenatal proof is usually only available if the fetus dies. If the fetus recovers before birth, IgM antibodies will be present in the cord blood. The diagnosis of neonatal congenital leptospirosis involves the same methods as used in adults.

8.1.2. Clinical Laboratory Findings. Laboratory tests, other than bacteriologic or serological, may help support the clinical diagnosis. In the absence of bleeding, the erythrocyte count and hemoglobin may be normal unless the patient is jaundiced, and then they are both reduced. Leukocytosis of 11–20,000/mm^3 (11–20 $\times$ 10^9/liter) is usual in icteric patients. The platelet count is frequently reduced, especially in severely jaundiced patients. The erythrocyte sedimentation rate is generally raised.

Blood urea and creatinine are frequently raised, at least transiently, even in mild cases. Liver function tests are useful diagnostically. The serum aminotransferases can be normal or mildly elevated. The aminotransferases are usually below 250 I.U./L and are seldom greater than 500 I.U./L. The serum bilirubin level is always raised in icteric or preicteric leptospirosis. The combination of raised blood urea, raised direct bilirubin, and normal or slightly elevated aminotransferases serves to differentiate leptospirosis from hepatitis in which the serum aminotransferases are always significantly elevated.[41]

Proteinuria is a constant but sometimes transient finding, even in mild cases. In severe cases, or wherever there is renal failure, granular and hyaline casts will be found in urine, together with bile.

Examination of CSF reveals a leukocytosis and increased protein with normal glucose.

8.1.3. Prognosis. Recovery is usually complete within a few weeks in mild-type patients. However, residual depression, irritability, and psychosis have been reported, lasting up to 6 months and preventing a return to normal life and work. Occasional reports of prolonged headache and backache following mild leptospirosis are hard to evaluate. Patients who survive the severe type of leptospirosis usually show no further signs of illness within a few weeks of full recovery of function in vital organs. Prolonged neurological symptoms and signs have been recorded, but in some of these patients there is room for doubt about the validity of the original diagnosis. In the severe disease mortality ranges from 5 to 25%.

8.1.4. Treatment. Penicillin (or erythromycin in allergic patients) is the recommended treatment of choice as early as possible in the illness.[38] Doxycycline [83] or a third-generation cephalosporin are also used.[97,117] Given on the first day or two in patients diagnosed in the acute stage of infection, it can abort the symptoms and progress of either the mild or the severe form abruptly, so that the patient is frequently clinically well within 24 h.

Antibiotics given in the late ("tissue") stages may make no difference to the outcome,[30,40] because the disease process is then a consequence of established lesions, the leptospires usually having been eliminated by the patient's development of antibodies. However, most clinicians would give patients the benefit of the doubt and administer intravenous penicillin, cefotaxime, or ceftriaxone. A Jarisch–Herxheimer reaction (temporary aggravation of symptoms and fever) may occur when antibiotic treatment is commenced in well-advanced patients,[54,126] but this should not prevent the initiation of antibiotic treatment.[138]

In severe cases, symptomatic and supportive systemic treatment is required for hemorrhage and shock. Meticulous

attention to fluid and electrolyte balance is critical. Renal failure may be treated by peritoneal dialysis, hemodialysis or continuous venovenous hemofiltration. There is no specific therapy for the hepatic insufficiency. Patients with arrhythmias and myocarditis should be monitored in an intensive care environment and the various arrhythmias treated. Severe pulmonary hemorrhage may require assisted positive pressure ventilation.

8.2. Diagnosis

8.2.1. Clinical Diagnosis. The clinical features in early leptospirosis of either the mild or the severe type are not pathognomonic and give no indication of the diagnosis to an inexperienced clinician. In areas where the disease is common and has been recognized using laboratory confirmation, fresh cases can be diagnosed with a high degree of accuracy, provided that there is not a concomitant outbreak of a similarly presenting disease at that time e.g., dengue fever.[73,106]

Epidemiological factors such as occupational or domestic exposure to risk of infection from carrier animals, recent exposure to fresh water with immersion, or recent travel will influence the interpretation of clinical findings, pending results of confirmatory laboratory tests.

8.2.2. Laboratory Bacteriologic Diagnosis. The principles of diagnosis are isolation of leptospires from blood, urine, CSF, or other sites during the acute illness, demonstration of leptospires in tissues and body fluids, and serological tests. Because of the low index of suspicion combined with the perceived difficulty of culturing leptospires, most cases are diagnosed by serology.

8.2.2.1. Diagnosis by Direct Detection of Leptospires. Leptospires can be seen occasionally in body fluids by dark-field illumination. Inexpert observers looking at body fluids can mistake fibrin threads for leptospires. Dark-field microscopy is neither sensitive nor specific enough for diagnostic use.[131] Leptospires may also be identified directly by staining of smears, or of frozen or wax-embedded histological sections of autopsy or biopsy material. Molecular diagnosis of leptospirosis is discussed above.

8.2.2.2. Culture. Diagnostic cultures are usually made from specimens of blood, urine, CSF, other body fluids and exudates, and occasionally from tissues obtained at biopsy or autopsy. Tween–albumin fluid culture media such as EMJH are most generally useful, because more exacting strains grow better in it on primary isolation. Special media are required for primary isolation and growth of fastidious pathogens.

Blood cultures are made by inoculating blood drawn aseptically by venipuncture directly into culture media. Inoculation of several tubes, each containing 5–10 ml of medium, with one or two drops of blood per tube (up to 250 μl per tube), is recommended. The blood should be diluted in the culture medium by a factor ofat least one in ten in order to dilute antileptospiral activity in the blood. If blood cannot be inoculated at the bedside, it can be collected in a sterile ammonium oxalate or heparin anticoagulant tube or even as a last resort in a plain tube and transported to a laboratory, where the blood or serum can be inoculated into medium. If possible, multiple cultures should be prepared from each specimen. Penicillinase and sodium polyanetholesulfonate (SPS) can be added if required. The inoculated media are incubated at 30°C and examined for growth by dark-field microscopy daily for the first week and weekly thereafter, up to 3 months, and ideally they should be subcultured weekly. The value of blood culture is definitive identification of the etiological agent rather than speed. Isolates may be identified by serological or molecular methods as described above.

Culture of urine requires an alkalinized fresh midstream or catheter specimen taken with care to minimize contamination. A selective medium containing 100 μg/ml 5-fluorouracil as an inhibitor of contaminant microorganisms and semisolid medium containing 0.1% agar are recommended, using at least a tenfold dilution of urine in the medium. Multiple cultures should be prepared from each specimen.

Leptospires may be found in CSF in the first 10 days of illness and can be cultured from it by inoculating 0.1–0.5 ml into 5–10 ml of fluid or semisolid culture medium.

8.2.2.3. Serological Diagnosis. Even if cultures are set up, recovery of leptospires requires weeks, rather than days, of incubation. Moreover, the diagnosis of leptospirosis is often not suspected, or the patient does not seek medical attention until the illness is well advanced, when leptospires be difficult to recover. Thus, serological tests may be the most reliable means by which a retrospective, if not concurrent, diagnosis can be made.

Both IgM detection by ELISA and detection of agglutinating antibodies by MAT are useful for diagnosis. Serum specimens for diagnosis by serological tests should be taken immediately at the outset of investigations, preferably on the first day of illness, and tested as soon as possible. A further specimen should be taken on the fifth to seventh days, and further tests if required on the 10th and 12th days. IgM antibodies are detectable 5–7 days after onset of symptoms, thus sensitivity of IgM detection is low during the first week of symptoms, rising rapidly after that.[32] Early in the illness, detection of IgM is significantly more sensitive than the MAT. A positive IgM should be followed by MAT testing of acute and convalescent specimens for confirmation by rising antibody titers.

8.2.3. Differential Diagnosis. At the onset of either mild or severe leptospirosis, there is little to distinguish it from any other acute febrile illness. The very severe headache, fever, and myalgia are characteristic, but at this stage they may be confused with severe influenza, poliomyelitis, dengue, Q fever, and viral meningitis. The main differentiating criteria in leptospirosis are raised blood creatinine and urea, leukocytosis, and in some cases, an early rise in titer of leptospiral antibodies. At a later stage the main differential diagnoses are with viral meningitis and acute abdominal emergencies in mild-type leptospirosis and with malaria, blackwater fever, viral meningitis, hepatitis, yellow fever, hemorrhagic fever with renal syndrome (HFRS) and acute glomerulonephritis in severe leptospirosis. The main diagnostic criteria are high serum bilirubin with low or normal levels of serum aminotransferases, thrombocytopenia, and renal insufficiency with significant and rising titers of specific antileptospiral antibodies. The isolation and identification of leptospires from blood, urine, or CSF, or a positive PCR assay, confirm the diagnosis. At autopsy, differential diagnosis depends on characteristic pathological changes of jaundice and widespread hemorrhages with pale, enlarged hemorrhagic kidneys showing histological evidence of renal tubular degeneration and hemorrhage, liver cell degeneration, myocarditis, and pulmonary hemorrhages. Postmortem serum specimens may be tested for antibodies. PCR and IHC testing of autopsy specimens are sensitive, particularly in patients who die of fulminant disease before they seroconvert.[17]

In all patients, an epidemiological history of occupational or accidental exposure to risk and animal contact or travel should increase the index of suspicion and indicate the need for specific diagnostic tests.

9. Control and Prevention

9.1. General Concepts

As is the case in all zoonoses, control of leptospirosis in humans is complex, because the primary sources of the disease are in animals, in some of which control is impossible. Regulation if not elimination of leptospirosis may be feasible in livestock and domestic animals, even though carriers are clinically normal and shedders hard to detect, but it cannot be envisaged in wildlife. Control is further complicated by the general and nonspecific clinical picture of mild or early severe leptospirosis, indistinguishable from a variety of other incapacitating fevers common in tropical areas, so that an animal source is often not suspected. The essentials of control and prevention lie in awareness and recognition of the disease and in containment in livestock and domestic sources.

Awareness and recognition require a knowledge of epidemiological facts acquired by selected surveillance, reporting, and notification, which in turn need specialized diagnostic laboratory services and education of physicians, veterinarians, administrators, and the public. Although containment of animal sources may be seen as a veterinary public health and hygiene problem, it is essential to the protection of humans. The main measures are veterinary surveillance, treatment, and prevention; occupational hygiene; rodent control; engineering; laboratory safety; and evaluation of preventive measures. In addition, containment extends to the prevention of human disease by the judicious avoidance of risk, by immunization, and by chemoprophylaxis.

9.1.1. Education, Laboratory Services, and Notification. Ordinary routine microbiological diagnostic laboratories are not usually equipped with skilled staff and specialized techniques for leptospirosis. If specialized diagnosis is not available locally, there should be a central leptospirosis laboratory available for referral of specimens and consultation. Reference laboratories staffed by experts are required only on a regional international basis for stock cultures, standard sera, research, education, and consultation. New methods of molecular diagnosis may make diagnostic facilities more readily available.

Evaluation of diagnostic laboratory results provides a useful form of surveillance. In addition, prevalence surveys for antibodies in selected groups can reveal problems or evaluate control measures. The most effective form of information gathering should be notification by health practitioners. Usually, this is of limited value because of inadequate diagnosis, failure to use appropriate laboratory tests, and failure to notify. Statistics of practitioner notifications frequently indicate an incidence much lower than diagnosed by laboratories.

9.1.2. Control Measures in Animals

9.1.2.1. Rodent Control. Removal of rodents from the domestic environment effectively reduces the infection rate of leptospirosis. Standard measures include control of litter and garbage disposal, protecting food sources, trapping, and poisoning. When they share the environment with crops, precautions can be taken, such as burning off sugarcane before cutting by hand or mechanization of cutting.[50]

9.1.2.2. Environmental Control. Any measure to reduce the load of leptospires in soil, water, or mud will reduce the risk to humans. Simple drainage can prevent seepage of urine into waterlogged soils or yards where livestock are herded. Environmental engineering can also increase the risk by flooding previously dry areas.

9.1.2.3. Immunization, Chemotherapy, and Culling of Livestock. Excreter rates in cattle and pig herds frequently reach levels of 65–90%. If shedders can be identified and removed from herds, the risks to the herd and to attendant humans can be reduced, provided the remaining animals are immunized, the herd is restocked with immunized animals, fresh excreter animals are not introduced, and the herd is protected from fresh infection from environmental sources. Immunization alone can reduce both animal and indirectly human leptospirosis, but it is hard to evaluate, because most studies have been specially set up under artificial conditions accompanied by education and improvements in occupational hygiene and farming practice. Culling or vaccination is both expensive and their use by small holding farmers will depend on the relative values and costs of animals, vaccines, and health (or illness). The associated costs of transport, skilled attention, and equipment may also be relatively high. The control of leptospirosis in stud stock, where leptospires may be found in semen samples used for artificial insemination, requires special methods for testing and transport of semen.

9.1.3. Treatment of the Carrier State. Carrier animals have been cured by streptomycin treatment. The cost is relatively high and the results so difficult to evaluate in field conditions that it is considered impractical for general use.[43,56]

Humans do not become chronic carriers or excreters for any length of time. Elementary domestic, personal, and hospital hygiene ensures little risk from patients and convalescents still excreting leptospires. Urine specimens from convalescent patients are not recognized sources of laboratory infection, but care should be taken nonetheless.

9.1.4. Occupational Hygiene. Preventive measures for people who cannot avoid exposure to risk are centered around methods for physical protection in their work or leisure. Waterproof footwear, watershedding aprons worn outside and over the boots or other clothing, and clear plastic facemasks have all been recommended for milkers and slaughterhouse workers. In hot climates or working conditions, impermeable protective clothing is uncomfortable and poorly accepted. Nevertheless, rubber boots provide obvious protection. Wearing of gloves by field workers, such as cane cutters, is also protective. Most infections appear to follow penetration of cuts and damaged or abraded skin by leptospires. It is therefore important to protect such injuries, but often not practicable to do so in daily working conditions.

Good drainage of floors and herding yards and control of rodent infestation in food preparation areas are important occupational hygienic measures, but no measures will be effective unless the people at risk understand the disease, the means of infection, and the need for precautions so that they are prepared to implement recommended practices. The risk for field workers can be reduced by attempting to control the environmental hazards.

9.2. Antimicrobial Prophylaxis

Chemoprophylaxis with doxycycline has been used to prevent leptospirosis in soldiers exposed to jungle conditions[119] and could be used similarly to protect anyone exposed to a significant risk for a brief defined period. People in such categories could include those about to undertake rafting or canoeing trips, cavers, grain farmers and workers at harvest time when risks are serious for a short period only, and field ecologists trapping small wild rodents. Doxycycline prophylaxis of large populations after severe flooding has not been shown to be effective for preventing infection,[57,108] but may reduce the incidence of clinical disease.

9.3. Immunization

Immunization of humans and animals with killed cultures of leptospires has been used almost since leptospires were discovered. Vaccines are serovar specific and currently administered subcutaneously in two or more doses 2 or 4 weeks apart and repeated annually. Vaccines for human use are licensed in France, Cuba, and several Asian countries.

Local reactions of pain, redness, and swelling ranging from mild to very severe occur, especially on revaccination. Washed or whole culture vaccines have been employed in several countries, mainly in Asia, where the risks to life and health from severe leptospirosis could justify the side effects of the vaccine.

10. Unresolved Problems

There is a series of interconnected questions that need answers before great improvements in control of leptospirosis can be achieved. Neither the duration of postinfection immunity nor its specificity is known. Conventional wisdom based mainly on animal experimentation states that immunity is specific for antigens that are serovar specific, or at best shared within a serogroup. Is there any underlying genus- or species-specific immunity in humans mediating a level of resistance to all serovars of pathogenic leptospires?

There are genetic differences in the susceptibility of pigs to primary infection. At least one study from an outbreak suggests that human susceptibility may be under genetic control.[76] Can this study be generalized, and if so, are the differences reflected in the response to particular serovars or to all serovars, and thus to the pathogenicity of particular serovars for humans?

There are over 250 serovars described among the pathogenic leptospires, based on antigenic surface characteristics mediated by LPS-related polysaccharide epitopes. Although part of the LPS gene has been characterized, the genetic basis for serovar specificity and variation is still unknown. This problem becomes acutely relevant with the recognition that the various species of *Leptospira* each contain serovars also found in other species; that is, serovar specificity overlaps the genetic groupings. Moroever, mosaicism within several genes suggests horizontal transfer.[60] The effects of these observations on classification of leptospires have implications for diagnosis, epidemiology, and prognosis. Many more isolates need to be genotyped, but to do this will require more easily applied methods.

Rapid bedside tests are required that will enable a clinician to identify whether or not a patient has leptospirosis, and if so, which serovar, often at a time in the illness before antibodies have appeared in blood. Direct tests for leptospires or their antigens would overcome the serious difficulty of evaluating the significance of a positive serological test in a patient in an endemic area where most of the population have antibodies, even residual IgM, to one or more serovars, or where the patient may have residual antibodies from an infection with a serovar unrelated to the present illness. Similar direct diagnostic methods would allow easier and more positive detection of carrier animals than is possible now.

Most people at risk from leptospirosis live in developing countries without ready access to specialized diagnostic or treatment facilities. There is seldom a capacity for new scientific research into the problems identified here. On the other hand, leptospirosis is rarely diagnosed in urban agglomerations in developed countries where most researchers and institutes capable of high technology are found and there are relatively few among them interested in leptospirosis. Knowledge and research in leptospirosis are thus not available in most developed countries, to which developing countries turn for scientific assistance. The situation is aggravated when inadequate statistics in developed countries indicate a spuriously low rate of leptospirosis, leading to an impression that the disease is nonexistent or unimportant and does not justify allocation of funds for the maintenance of existing research and surveillance or for new studies toward solutions to the problems outlined above.

11. References

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CHAPTER 22

Listeria monocytogenes Infections

Donald Armstrong and Donald B. Louria

1. Introduction

Listeria monocytogenes has been recognized as a human pathogen since the turn of the nineteenth century. Investigations of listeriosis epidemics in North America and Europe over the past 25 years have confirmed a long-held suspicion that listeriosis is a foodborne disease.[1–7] *L. monocytogenes* infections resulting in invasive disease occur particularly often in the immunocompromised host, specifically those in whom the T-helper cell−mononuclear phagocyte arm of the immune defense system is altered. Severe disease also occurs in the very young and in older persons as well as in patients with neoplastic disease and in recipients of organ transplants. Alcoholism, cirrhosis, and diabetes mellitus are also frequently associated risk factors, as are adrenocorticosteroid therapy for underlying disorders such as collagen vascular disease or inflammatory bowel disease and treatment of certain chronic diseases with tumor necrosis factor alpha (TNF_alpha)-neutralizing agents.[8–11] Although not a common opportunistic infection among patients with advanced human immunodeficiency virus (HIV) infections, listeriosis has been shown to be at least 100 times as frequent in acquired immunodeficiency syndrome (AIDS) patients than in the general population.[12,13] *L. monocytogenes* has a predilection for pregnant women, with potentially lethal consequences for the fetus in utero and the newborn. Normal hosts are not entirely resistant to invasive infections, and the occurrence of listeriosis in such individuals should not warrant an exhaustive search for an underlying but yet undiagnosed immunosuppressive disorder.

In immunocompromised and elderly adults (nonperinatal listeriosis), the clinical presentation is usually sepsis or meningitis, or a more subtle nonmeningitis central nervous system (CNS) infection such as meningoencephalitis, cerebritis, or brain abscess. While these severe forms of listeriosis have been well described, recent investigations of point-source foodborne outbreaks of listeriosis have further confirmed the clinical entity of a milder, noninvasive gastrointestinal illness characterized by fever, nausea, vomiting, diarrhea, headache, and musculoskeletal symptoms, indistinguishable from other common presentations of food poisoning.[5–7,14,15] Such an illness may be a prodrome of a more severe, invasive infection, especially in groups known to be at high risk for invasive disease. Table 1 contains a categorization of *L. monocytogenes* infections.

In pregnant women (perinatal listeriosis), infection may present as a flu-like illness with fever, headache, and myalgia and progress to amnionitis and premature labor.[16] The corresponding intrauterine infection is usually apparent at birth or within the first few days of life. This congenital infection, granulomatosis infantisepticum, is characterized by a severe, often fatal, disseminated, multiorgan infection. In contrast, newborns who acquire listeriosis at the time of delivery or shortly thereafter are usually full term and the product of an uncomplicated pregnancy. These infants acquire *L. monocytogenes* at delivery when exposed to a contaminated birth canal or via nosocomial transmission in the hospital.[17–21] In this group, the clinical presentation is more likely to be meningitis than overwhelming sepsis.[20,21]

Sporadic cases are the rule, but outbreaks have been well described due to an expanding variety of contaminated foods including cole slaw, corn, cheese, butter, milk, smoked fish, and other seafood products and processed meats.[1,3,22,23] The number of outbreaks recorded has been small and predominantly in North America and Europe. However, given the nature of the organism, there is no reason

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Table 1. ***Listeria monocytogenes*** **Infection**

Host	Illness	Type of infection
Gravid woman	"Flu-like" syndrome	Bacteremia and/or chorioamnionitis
Neonate	Disseminated, lethal septic disease	*In utero*, widely disseminated, multiorgan disease; "granulomatosis infantiseptica"
	Sepsis and/or meningitis	Postpartum bacteremia (may or may not progress to meningitis)
Adult (often immuno-compromised)	Sepsis and/or meningitis	Bacteremia (may or may not progress to meningitis)
	Meningoencephalitis, rhomboencephalitis, cerebritis or brain abscess	Subacute cerebritis and/or meningitis (usually does not progress to brain abscess)
Any age	Focal infection[a]	Resulting from bacteremia or direct inoculation (skin)
	Gastroenteritis	Mild gastroenteritis following recent exposure to contaminated food product, usually self-limited

[a]Includes skin, ocular, lymph node, endocarditis, arteritis, artificial valve and stent infection, pneumonia, osteomyelitis, prosthetic joint infection, spinal or brain abscess, peritonitis, cholecystitis, and hepatitis.

to believe that the disease is limited to these regions. Rather, listeriosis may be occurring but not detected due to difficulties with bacterial isolation and identification.[22] Nosocomial outbreaks have also been described in newborn nurseries and renal transplant units.[17–20] Since pregnant women appear to be highly susceptible to this infection, the occurrence of listeriosis in pregnant women should alert public health officials to the possibility of community-wide exposure to *L. monocytogenes*, as it can be assumed that many more people are exposed than develop disease.

2. Historical Background

L. monocytogenes was first isolated from necrotic lesions in the liver of rabbits in 1919, and named *Bacillus hepatitis*.[24] This was the same year that French clinicians isolated and preserved a "diphtheroid" from spinal fluid cultures of a patient with meningitis, subsequently identified as *L. monocytogenes*.[25] In 1924, this organism was named *Bacterium monocytogenes* for the monocytosis that is associated with systemic illness in an outbreak among laboratory rabbits in England.[26,27] The etiologic agent of an epizootic among wild gerbils from South Africa was identified as *Listerella hepatolytica* in honor of the father of antisepsis, Joseph Lister.[28] The organism was named *L. monocytogenes* in 1940.[29,30] Despite the name of the organism, monocytosis is an unusual feature of human infections.

Initially recognized as an infection of animals, *L. monocytogenes* was considered a veterinary disease, causing epizootics in sheep and cattle, with manifestations of abortions and basilar meningoencephalitis ("circling disease") in mammals. In humans, the association of a *Listeria* infection in pregnancy and severe disease in the fetus was first described in 1936,[31] and the association between *L. monocytogenes* and neoplastic disease was first reported by Louria and colleagues in 1967.[32] Since then, confirmatory reports have further identified a range of underlying conditions that increase the risk of listeriosis. These include renal and other organ solid transplant patients,[33] alcoholism and cirrhosis,[34] diabetes mellitus, and conditions that are typified by dysfunctional cellular immunity, including persons on immunosuppressive doses of adrenocorticosteroids,[35–40] and those with HIV infection.[12,13]Among those with malignancies, the majority suffer from hematologic malignancies that are characterized by impairment of cellular immunity, in particular Hodgkin's disease, non-Hodgkin lymphoma, and lymphatic leukemias.[39,40] Among transplant recipients, cytomegalic virus infection may also be a predisposing factor in *Listeria* superinfection.[41]

Well-characterized food outbreaks have been reported.[1–6] Clusters of cases have also occurred among immunocompromised hosts and in hospital settings where the source of nosocomial transmission was not always identified.[16–18,33,36,42–44] A well-documented investigation of an outbreak of listeriosis among newborns at a hospital in Costa Rica linked to aspiration of mineral oil used to bathe infants provided overwhelming evidence that transmission is not limited to the ingestion of contaminated foods.[19] In one study, contact with a single caregiver and inadequate handwashing were implicated.[45]

Gastrointestinal carriage of *L. monocytogenes* without signs or symptoms of systemic listeriosis has been well documented. Prevalence rates in community studies range from 1–5% to 5–29% (the latter in healthy abattoir workers and high-risk groups).[46,47] Recent studies have also documented the increased rate of asymptomatic stool carriage in up to one-fifth of the household contacts of patients with listeriosis. However, despite these carriage rates, secondary cases within a household have not been documented. This

reinforces the importance of the susceptibility of the host exposed to this organism, and constitutes additional evidence that exposure greatly exceeds observed disease.[48–53] Rates ofintestinal carriage of *L. monocytogenes* by pregnant women may reach 40%.[49] Gastric acidity protects against *L. monocytogenes* colonization; stool carriage rates increase substantially in patients treated with H_2-antagonists.[54]

3. Methodology

Since *L. monocytogenes* infections have not been reportable in most counties until recently, including the United States, accurate figures on incidence are not generally available. In an effort to improve the accuracy of surveillance data in the United States, in 1986, the Council of State and Territorial Epidemiologists (CSTE) recommended that listeriosis be made a reportable disease and highlighted the importance of clinical microbiology laboratories as an important source of case reports. Prior to this, the most comprehensive US study was based on hospital discharge data from hospitals participating in the Professional Activities Study (PAS) of the Commission on Professional and Hospital Activities (CPHA), a nonprofit, nongovernmental hospital discharge data system.[3] The data set was generated from the data bank of the CPHA-PAS-participating hospitals using the discharge diagnosis of listeriosis during 1980, 1981, and 1982. The number of hospitals participating in PAS ranged from 1354 (1980) to 1283 (1982), with a mean of 1309 hospitals per year. These hospitals represented 22–23% of all short-term, nonfederal hospitals in the United States and included 27–29% of all US discharges from such hospitals. For each state, total hospitalizations for listeriosis were projected by dividing the number of cases from participating hospitals in a given state by the proportion of all the states' discharges that were from PAS hospitals. A more accurate measure of listeriosis in the United States has been reported by CDC investigators' active surveillance project that began in 1986 and collected data from biweekly communication with infection control coordinators at all acute care hospitals in a several state area (see below).[3,55]

Following four large foodborne outbreaks of listeriosis in North America in the early 1980s, the CDC initiated an active surveillance project with six geographically distinct state and county health departments covering a total population base of approximately 34 million persons, representing over 14% of the US population.[3] In these surveillance areas (Missouri, New Jersey, Oklahoma, Tennessee, Washington, and Los Angeles County), all patients in whom *L. monocytogenes* was isolated from cultures of normally sterile body sites were reported to the state's or county's Listeriosis Study Group coordinator via biweekly telephone reports by hospital-based contacts (usually the infection control nurse) at each of the 666 acute-care hospitals. A single-page case report form with demographic and clinical information was completed for each case. The clinical isolates of *L. monocytogenes* from these case patients were confirmed and serotyping and molecular fingerprinting by multilocus enzyme electrophoresis were performed to further characterize these clinical isolates. This multi-state active surveillance system has been maintained, with some modifications, since 1986, replacing the passive reporting system based on data from hospital discharge diagnoses.[55] In 1996, the Foodborne Diseases Active Surveillance Network (FoodNet) of CDC's Emerging Infections Program began collecting data on foodborne illnesses, first in five and subsequently in ten states (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee). This intensive, ongoing assessment of listeriosis in the United States over time has supplied a measure of the impact that educational efforts and the concomitant tightening of regulations of the food industry have had on the health of the public.

3.1. Sources of Mortality Data

Sources of mortality data include case reports, descriptions of outbreaks, reviews, and recent epidemiological surveillance and surveys. Mortality rates vary considerably with the age and underlying medical condition of the host. At least 20% of pregnancies complicated by listeriosis result in intrauterine fetal death, but only rarely does this infection persist in the mother following delivery. However, the full impact of listeriosis in pregnancy may be underestimated, as suggested by a French study where researchers cultured *L. monocytogenes* from the placentas and/or fetuses of 1.6% of all pregnancies that resulted in preterm labor or spontaneous abortion.[56] In the severest form of congenital infection, granulomatosis infantisepticum, mortality has ranged from 33 to 100%.[48,57,58] In contrast, late-onset neonatal cases (onset between 8 and 28 days of life) are more likely to present with meningitis than sepsis and have a more favorable outcome compared with early-onset neonatal cases.[20,21`] In the largest North American epidemic of listeriosis in Los Angeles in 1985, there was a 63% case fatality rate for early-onset neonatal cases, in contrast with a 37% case fatality rate among late-onset neonatal cases.[4]

The CDC's 1986 *Listeria* surveillance project documented that the case fatality rate in patients with sepsis (40%) was higher than that of patients with meningitis

(27%), although the difference was not statistically different and may have been affected by a misclassification bias due to a lack of cerebrospinal fluid (CSF) cultures.[3] In invasive disease, overall mortality rates have been estimated as 19–35%, with the rate increasing with increasing age.[22,55,59]

3.2. Sources of Morbidity Data

Since reported listeriosis cases are usually due to severe invasive disease, patients are usually hospitalized. Therefore, sources of morbidity data are similar to those of mortality data. Among 27 states participating in the National Bacterial Meningitis Surveillance Study, there were 265 cases of *Listeria* meningitis between 1978 and 1981.[60] In some regions, *Listeria* was reported as the second most common cause of neonatal meningitis (after Group B streptococcus) and the second most common cause of meningitis in individuals over 60 years old (after *Streptococcus pneumoniae*). Sources of morbidity data have also been obtained from epidemiological surveillance and from investigations of outbreaks of listeriosis.[57,58,61–66] The recent documentation of a mild, self-limited gastrointestinal syndrome associated with the ingestion of *Listeria*-contaminated food products highlights the fact that additional cases of *Listeria* infection in the community almost certainly go undetected.[5–7,14,15]

3.3. Surveys

The detection and investigation of recent epidemics underscore the need for improved surveillance of listeriosis. Even though the attack rate of epidemic listeriosis may be four- to six-fold higher than the background rate,[67] since the absolute attack rate during an epidemic may be as low as 0.8–2.0 per 10^5 population, an ongoing epidemic may not be observed in a community, especially if patients are being diagnosed and treated at several hospitals in the community. In the epidemic in Los Angeles County in 1985 associated with a contaminated Mexican-type soft cheese, the outbreak was detected by an alert infection control nurse in the county women's hospital that provided care for the largest segment of the Hispanic population. It was only after an unusual number of cases occurred in this hospital that Los Angeles County health officials were notified and the full extent of the community-wide outbreak was revealed.[4] Had the 142 cases in Los Angeles County been distributed throughout the hospitals in the county, it is unlikely that the few patients at each hospital would have been other than a clinical curiosity.

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organism. Isolation and identification of the organism are not difficult as long as laboratory personnel recognize the importance of scrutinizing gram-positive, diphtheroid-like organisms for the possibility of being *L. monocytogenes*.[32,68] *L. monocytogenes* is motile and hemolytic, unlike other "diphtheroids." The organism's tumbling motility is best seen at room temperature and characteristic hemolysis may be minimal but enhanced with passage. Presumptive identification can be made based on these properties, while final identification requires an additional 24 hours.

In cases of meningitis, the organism is seen on Gram stain in only 10% of cases. The absence of organisms on Gram stain in the presence of a pleocytosis in the appropriate host should strongly raise the possibility of *L. monocytogenes*. When attempting to isolate the organism from food, stool, or environmental sources, holding specimens at 4°C has resulted in improved yields, but this "cold enrichment" technique has not been demonstrated to be more efficacious with clinical specimens such as CSF, blood, or others.[68] The cold enrichment method has been improved upon by the United States Department of Agriculture's (USDA) culture method.[69,70] Because of concern of *Listeria* contamination in the food industry, a variety of rapid diagnostic techniques have been developed for its detection in food and environmental samples. These have included spectroscopy,[71] genetic probes based on the listeriolysin O gene sequences or species-specific rRNA sequences,[72,73] other genetic probes,[74] monoclinal antibodies to cell surface antigens,[75] and a variety of polymerase chain (PCR)-based methods.[74,76,77,78] In the future, additional rapid identification tests will be available, based primarily on PCR methodology, but also probably using nanotechnology.

Of the seven species of *Listeria, L. monocytogenes* is considered to be the principal human pathogen, although there are case reports of invasive listeriosis caused by species other than *L. monocytogenes*, including *L. innocua*[34,79–81] There are 11 serotypes of *L. monocytogenes*, differentiated on the basis of agglutination of somatic (O) and flagellar (H) antigens.[52,68] Serotypes 1/2a, 1/2b, and 4b account for over 90% of all isolates, although the distribution of these serotypes may vary by geographic region.[3,55,63,68] Other than the fact that the majority of *Listeria* infections are caused by these three serotypes, no clinical differences in disease presentation or severity have been demonstrated among the various serotypes.

Since just three principal serotypes cause most human disease, serotyping systems have been of limited use in epidemiological investigations, including

outbreaks and attempts to link sporadic cases of listeriosis to an implicated food item or environmental specimen. More discriminatory subtyping techniques include phage typing [63,62] pulse-gel electrophoresis,[83–88] multilocus enzyme electrophoresis,[89–92] restriction fragment length polymorphism (RFLP),[93–95] multi-virulence locus sequence typing,[96] ribosomal DNA fingerprinting (ribotyping),[85,87,97–99] and PCR-based randomly amplified polymorphic DNA patterns (RAPD) or repetitive element sequence analysis,[98,100–102] and mixed genomic microarrays.[103] These increasingly sophisticated techniques are powerful tools in epidemiologic assessment of both outbreaks and sporadic cases.

3.4.2. Serological and Immunological Diagnostic Methods. *L. monocytogenes* has several antigens that cross-react with other gram-positive organisms; therefore, false-positive serological results may occur. Further, gastrointestinal carriage studies and the recently described mild gastrointestinal manifestations of *L. monocytogenes* infection suggest that exposure may be widespread.[5–7,14,15] Therefore, *Listeria* agglutination titers may be detected in persons without evidence of having experienced invasive listeriosis. Forexample, 40% of abattoir-associated personnel in India (in the absence of clinical disease) were found to have anti-*Listeria* antibodies (Igm and IgG) using a dot-ELISA technique.[46] Earlier attempts at serological diagnosis were limited by the lack of sensitivity and specificity of the available assays, and false-negative serological tests were observed in patients with culture-proven listeriosis, especially in newborns and immunosuppressed patients,[1,68,80,104,105] however, the use of an improved antilisteriolysin O assay as part of a preliminary outbreak investigation shows promise.[106] Like many serological tests, this one offers little assistance in the clinical management of a patient.

4. Biological Characteristics of the Organism

Its precise ecological niche is unknown, but *L. monocytogenes* has been isolated from a wide variety of environmental sources. Its survival in the environment was best documented from quantitative bacterial counts demonstrating persistence of viable organisms for at least 2 months in various agricultural settings.[107] Carriage studies have repeatedly demonstrated that the organism can survive in the human gastrointestinal tract.[48–53] In addition, it has been shown to live in the gastrointestinal and genital tract of a variety of animals ranging from shellfish to birds and most mammals. Its predilection for immunocompromised hosts, including pregnant women, attests to its low pathogenicity in immunologically normal individuals.

5. Descriptive Epidemiology

Initially described as a zoonosis, *L. monocytogenes* is responsible for abortion, mastitis, septicemia, meningitis, and encephalitis in cattle and sheep and is the etiologic agent of circling disease. Zoonotics in sheep and cattle have been attributed to poor-quality silage, a link that has been speculative and only rarely documented.[108,109]

Investigation of a series of outbreaks of human listeriosis since the early 1980s has clearly demonstrated that listeriosis is a foodborne disease. Although the bulk of human listeriosis occurs as individual "sporadic" cases rather than in the setting of an outbreak, investigations of such cases have continued to demonstrate that they often result from an encounter between a susceptible person and a *Listeria*-contaminated food item. The lessons learned from the investigation of epidemics regarding foods at high risk of *Listeria* contamination coupled with increased concern of food safety at a microbiological level is likely responsible for the marked decline in human listeriosis in the United States over the past decades.[55] After initially concentrating on dairy products, both FDA and USDA's Food Safety Inspection Service (FSIS) have broadened their surveillance for *Listeria*-contaminated products following a well-documented outbreak of listeriosis that was linked to a contaminated turkey frankfurter.[110–114] Following the epidemics in the early 1980s, these agencies ultimately developed a "zero tolerance" posture. In 1986, the first full year of the program, over 40 commercial food products were recalled or kept from being distributed due to *L. monocytogenes* contamination.[3] Since then, over 30 food recalls have been initiated each year, including ready-to-eat delicatessen meats and poultry products, prepared salads, and seafoods.[55,115]

5.1. Prevalence and Incidence

5.1.1. Asymptomatic Carriage. Epidemiological evidence that listeriosis is usually a foodborne infection is further strengthened by studies of asymptomatic gastrointestinal carriage. This has been well documented, although prevalence rates have varied in different studies as a function of the subject selection, sampling frame, and culture technique. However, since the identification of *L. monocytogenes* from cultures of samples contaminated with other bacteria can be difficult and since many of these carriage studies were conducted prior to the development of selective and sensitive

culture techniques, it cannot be certain that these rates reflect only pathological strains of *Listeria*. Nevertheless, these rates have ranged from 1–5% in community-based studies to 5–29% in healthy abattoir workers and poultry workers to 8–26% among household members of listeriosis patients, the latter presumably having similar exposure risks as the case patient, further evidence that exposure may greatly exceed observed disease.[48,51,52,115]. Indeed, an intensive 1-year study of 3 healthy volunteers (242–363 stool specimens) showed an overall 1.15% *L. monocytogenes* isolation rate, but evidence of 5–9 exposures to *L. monocytogenes* per person per year in the absence of clinical disease.[116]

5.1.2. Gastroenteritis. The isolation of *L. monocytogenes* from stool may be problematic. Population-based studies of listeriosis have focused on invasive disease with a case definition that depends on the recovery of *L. monocytogenes* from a normally sterile site. Therefore, the full burden of *L. monocytogenes* morbidity is also likely to have been underestimated. Mild gastrointestinal illness attributed to listeriosis has been frequently documented,[6,7,14,15] but it is difficult to distinguish from a variety of pathogens and conditions that are characterized by similar symptoms.

5.1.3. Invasive Infection. Despite the indirect evidence from food microbiology and gastrointestinal carriage studies that exposure to pathogenic *L. monocytogenes* is common, serious infection is *infrequent*. Infection occurs primarily among pregnant women and their neonates, hosts immunocompromised by their underlying illnesses or medications, and the elderly.[22,117] Prior to the CDC's active surveillance studies and the CSTE's recommendation for laboratory reporting of cases of listeriosis in 1986, estimates of the incidence of listeriosis depended on passive reporting systems or hospital discharge data. These methods were acknowledged to underestimate the true burden of disease and made it difficult for direct comparisons of incidence data collected in different ways.[53] The CDC's study of hospital discharge data between 1980 and 1982 estimated the annual incidence of listeriosis in the United States to be $0.36/10^5$, accounting for approximately 800 cases and over 150 deaths (case fatality rate, 19%). In contrast, in the CDC's more intensive 1986 active surveillance study incidence rates were nearly twice that estimated by hospital discharge data.[3] In Europe in 1986–1987, incidence rates ranged from $0.5/10^5$ in the United Kingdom to $1.1/10^5$ in France.[118,119] Whether these differences reflect true difference in incidence or differences in surveillance and reporting have not been assessed.

The CDC's active surveillance project identified 246 cases of listeriosis that occurred among residents of the surveillance areas in 1986; the overall incidence was $0.7/10^5$: 67 perinatal cases ($12.7/10^5$ live births) and 179 cases in nonpregnant adults (non-perinatal cases, incidence = $0.5/10^5$). From these data, it was estimated that in 1986 at least 1700 cases of listeriosis occurred in the United States annually, resulting in approximately 450 deaths. The incidence of listeriosis increases with advancing age; more than 80% of non-neonatal patients with listeriosis are over 50 years of age and over 40% are 70 years of age or more[3]. This age distributionappears to reflect both heightened risk due to immunologic deficits that accompany the aging process and the greater prevalence of underlying diseases that predispose to *Listeria*, as well as the use of *Listeria*-promoting medications to treat these underlying conditions.

In pregnancies complicated by *L. monocytogenes* infections that resulted in intrauterine fetal death, gestational age was not uniformly reported; however, the earliest documented gestational age reported was 11 weeks. Perinatal incidence was four times higher in Los Angeles ($24.3/10^5$) than in other areas ($5.2/10^5$ births, $p = 5 \times 10^{-5}$), and race-specific perinatal rates in Los Angeles were higher among blacks ($51.1/10^5$ births than among whites ($21.7/10^5$ births) or Hispanics ($22/10^5$ births), $p = 0.03$. The perinatal rate for whites and blacks in Los Angeles was higher than comparable race-specific rates in the other areas (whites = $10.7/10^5$ births, blacks $5.6/10^5$ live births, $p = 0.003$). The higher rates could not be attributed to maternal age or serotype distribution. Since this 1986 surveillance study occurred during the year following the well-publicized cheese-borne listeriosis epidemic in Los Angeles, it was speculated that clinicians may have had a heightened index of suspicion and increased the number of cultures they performed to rule out a *Listeria* infection.

In the CDC's 1986 surveillance project, 162 clinical isolates were serotyped. Three serotypes (1/2a, 1/2b, and 4b) accounted for nearly 95% of all strains: 30% were serotype 1/2a, 32% serotype 1/2b, and 33% were serotype 4b. The remaining nine strains were distributed among three additional serotypes: 3a, 3b, and 1/2c. These isolates were further subtyped by multilocus enzyme electrophoresis and found to be distributed among 37 distinct electrophoretic types, a method by which all strains can be typed.[89] This demonstrated both the diversity of the species and the promise of molecular biological techniques to clarify the epidemiology of listeriosis.[3]

Between 1996 and 2006, the CDC FoodNet surveillance population increased from 14.2 million persons (5% of the US population) in five states to 44.9 million persons (15% of the US population) in ten states. During that period, compared to the 1996–1998 baseline, the incidence of laboratory-confirmed *Listeria* cases has decreased by 34% to 0.31 per 100,000.[120] It is likely that multifaceted prevention efforts by industry, food regulatory agencies, and from widely disseminated consumer guidelines have been responsible for

this decline (Figure 2). In France between 1987 and 1997, an incidence reduction of 68% was observed, again attributed in large part to prevention efforts.[121]

Case fatality rates have varied considerably depending on age and extent of underlying disease of the populations studied. At one cancer center between 1955 and 1997, it was 39%.[37] Mead and colleagues calculated a 20% case fatality rate for invasive listeriosis based on 1997 US data.[122] Siegman-Igra and colleagues reviewed infectious disease reports of non-perinatal *Listeria*, a total of 1,149 cases published in the 1990s; case fatality rates ranged from 24 to 52%, averaging 36%. In six reports of perinatal listeriosis with a total of 494 cases combined intrauterine and postnatal case fatality rates ranged from 19 to 45%, averaging 33%.[123] In another review (of 222 cases), the perinatal case fatality rate was about 20%.[124]

Examination of CDC incidence and crude mortality data in the 2003–2006 time period suggests current case fatality rates area considerably lower than the 20%[120,125] reported by Mead and colleagues for 1997, almost all of whom were hospitalized. Lower current case fatality rates could be related to inclusion of more isolates from febrile gastroenteritis cases, more effective treatment, or better control of underlying diseases with resultant more robust host anti-*Listeria* immune mechanisms.

5.2. Epidemic Behavior and Contagiousness

Neonatal infections aside, the majority of listeriosis epidemics have implicated contaminated food items. Foods have included milk,[2,5] butter,[126] soft cheese,[4,127] cole slaw,[1]smoked fish, other seafood products,[84,128] ready to eat deli meats, frankfurters, and poultry products.[88,111,129,130] In 2002, a nine-state outbreak of listeriosis due to an unusual strain implicated turkey deli meat and was traced to two production facilities.[88] Contamination of dairy products may have occurred either from contaminated milk obtained from cows with *Listeria* mastitis or as a result of in-process or post-pasteurization contamination of products being prepared and packaged in *Listeria*-contaminated environments. Similarly, *Listeria* can contaminate meats and seafoods at anystage of the food production process. The cole slaw contamination was attributed to the local practice of fertilizing with sheep manure. Unfortunately, in this circumstance the source of manure was from a sheep herd that had apparently suffered a *Listeria* epizootic. Since the cabbage was kept in silos over the winter, enhancement of bacterial growth by an unintentional cold enrichment process was also considered as a contributory factor.

It is apparent that, in several of the community-wide outbreaks linked to contaminated food items, many more are exposed than become ill.[131] Carriage studies have shown elevated rates of asymptomatic carriage in household contacts of listeriosis patients, as well as members of the community at large, even among individuals considered to be at high risk of acquiring listeriosis.[37,49–52] Other than vertical transmission from a pregnant woman to her fetus, no cases have been linked to person-to-person transmission although outbreaks among neonates in hospital settings have raised suspicion about cross-contamination.[19,132–134] The descriptions of a milder, noninvasive form of listeriosis characterized predominantly by gastrointestinal symptoms have demonstrated a broader spectrum of illness; however, there is no evidence that differences in clinical presentation are linked to strain-specific differences among *L. monocytogenes* isolates. In contrast, although there are case reports of *Listeria* species other than *L. monocytogenes* causing disease, all strains of *L. monocytogenes* are considered to be potential pathogens. The reason why just 3 of the 11 serotypes are responsible for the bulk of disease remains unanswered.[3]

The infectious dose of *L. monocytogenes* in humans is not known, although it is likely that there is an interaction between the host susceptibility and the size of the inoculum. This is supported by feeding studies of steroid-treated mice, Sprague–Dawley rats with altered gastric acidity,[135,136] and feeding trials in nonhuman primates.[137,138] Nevertheless, since the bacterial counts of *Listeria*-contaminated food items may increase with prolonged shelf life and since differences in host defense mechanisms influence susceptibility to *L. monocytogenes*, a zero tolerance standard has been adopted by the food industry.[139] It has been proposed that infection may occur either after ingestion of a single large dose of *L. monocytogenes* or after repeated ingestions of smaller doses.[116]

5.3. Geographic Distribution

Surveillance studies, outbreak investigations, and case reports of listeriosis, coupled with its ubiquity in the environment, suggest that *L. monocytogenes* has a worldwide distribution. Differences in disease rates have been attributed to differences in surveillance and/or the likelihood of exposure to foods that may be contaminated.

5.4. Temporal Distribution

The incidence of listeriosis is generally believed to be higher in humans from the late spring to early fall.[3] In other

animals it appears more often in the winter. Presumably this is related to seasonal exposure to contaminated food or environmental sources, but this has not been adequately documented.

5.5. Age

The very young and very old have the highest incidence rates and mortality rates. Beyond the neonatal period, the incidence of listeriosis remains low until the *seventh* decade of life, when it increases with advancing age. More than 40% of cases occur in individuals 70 years of age or more.[3,55] In the immunosuppressed patient the age varies with the reason for immunosuppression and the age of onset of the underlying immunosuppressing condition (e.g., pregnancy, organ transplantation, and HIV infection).

5.6. Sex

Other than the predilection for pregnant women, there are no significant gender-specific differences in incidence, clinical presentation, or severity of disease.

5.7. Race

Differential rates of disease may be associated with food items to which some groups may be more likely to be exposed; however, there are no data to support differences in disease that are inherent to specific racial or ethnic groups.

5.8. Occupation

Other than a handful of case reports of cutaneous listeriosis among veterinarians, farmers, and ranchers, most of whom participated in the delivery of infected and aborted calves, there are no defined occupational risks for developing invasive listeriosis.[140,141] Groups with greater exposure to the organism may have higher gastrointestinal carriage rates, however, but there is no evidence that disease is more common.

5.9. Occurrence in Different Settings

In addition to community-wide foodborne epidemics, small outbreaks in organ transplant units and newborn nurseries have been described.[33,45,132–134,142]

5.10. Socioeconomic Factors

There are no known socioeconomic factors that affect risk of acquiring listeriosis.

5.11. Other Factors

Several reports have suggested that underlying gastrointestinal conditions such as reduced gastric acidity,[42,59,136] a concomitant gastrointestinal infection,[143,144] or a recent gastrointestinal procedure[145,146] may facilitate invasion and result in a systemic infection. Whether these represent true risk factors is a hypothesis that has yet to be adequately addressed.

6. Mechanisms and Routes of Transmission

The growing number of reports of sporadic listeriosis traced to a contaminated food product and the evidence of contaminated foods as the source of community-wide outbreaks have confirmed that listeriosis is a principally foodborne infection, although the well-documented epidemic of nosocomial listeriosis traced to contaminated mineral oil aspirated by newborn infants in the nursery demonstrates the epidemic potential in other settings.[19] However, given the ecology of this organism, it is likely that contaminated food products are ultimately linked to an infected animal, with the subsequent direct or indirect contamination of selected food products during preparation, processing, or packaging. The gastrointestinal reservoir offers the potential for person-to-person transmission via fecal–oral spread, but there is no evidence to support this hypothesis. As noted earlier, direct contact with this bacterium has resulted in focal infections in those attending the delivery of infected or aborted calves.[140,141]

7. Pathogenesis and Immunity

The investigation of outbreaks and well-documented point-sources of exposure to *Listeria*-contaminated foods has helped to clarify the incubation period of foodborne listeriosis and may range from as short as 2–3 days to 2–4 weeks.[4,15,129] In addition, there are also reports that suggest that it may be considerably longer, although documentation of a precise point of exposure limits generalizations of reports of protracted incubation periods.[4] Also, persons with known risk factors for listeriosis may be transient asymptomatic carriers and never develop invasive disease.[116,147]

Once it invades, the organism appears to reside in the protected environment of nonactivated macrophages where it is shielded from antibiotics, a feature that has important implications for antimicrobial therapy. An in vitro human enterocyte-like model suggests that *L. monocytogenes* is protected by intracellular vacuoles resulting from phagocytosis and that listerolysin O, a hemolysin with structural and physiological similarities to hemolysins of *S. pneumoniae* and *S. pyogenes*, constitutes one virulence factor whereby the intravacuolar bacteria can escape into the cytoplasm proper, an environment conducive to replication.[148–151] Only *L. monocytogenes* and *L. ivanovii*, but not other species of *Listeria*, produce this toxin.[149] In the 1960s, the importance of the mononuclear phagocyte in host resistance to *L. monocytogenes* was demonstrated.[152,153]

Both innate and acquired immunity appear critical in controlling invasive *Listeria* infections.[23,154–157] The former is important in the early stages; neutrophils, natural killer cells (secreting interferon gamma), and macrophages all appear to play a significant role. Subsequently acquired immunity develops, mediated primarily by CD8 T cells both by cytotoxic activity and by secretion of multiple cytokines, including interferon gamma (INF gamma) and tumor necrosis factor alpha (TNF alpha). The importance of TNF alpha in controlling *Listeria* infections provides a compelling explanation for the supervention of severe invasive *Listeria* infections in patients given TNF alpha blockers for the treatment of rheumatoid arthritis, Crohn's disease, or psoriasis.(8–11)

Antibody, complement, and CD4 T lymphocytes were, in the past, thought to play virtually no role in defense against *Listeria*. Recent evidence suggests that they do play a lesser role, though the importance of that role remains unsettled.[154–158]

In recent years, a great deal of research has elucidated the complexity of the host–*Listeria* relationship, the role of multiple *Listeria* components that aid its invasive and growth capacities, and the T-cell-dominated host defenses. The many elegant studies also make it clear that there is still a great deal to learn about the host–*Listeria* relationships.[23,154–157]

8. Patterns of Host Response

8.1. Patterns of Host Response

The response observed in histopathologic studies of both animal models and human disease varies from the production of a neutrophilic exudate to granulomas. This is reflected in the CSF pleocytosis, which varies from a predominantly neutrophilic to a predominantly mononuclear response. The investigation of foodborne epidemics and recent documentation of a mild form of listeriosis with predominantly gastrointestinal manifestations suggests that the gastrointestinal tract is the initial portal of entry; however, no gastrointestinal lesion has been consistently observed. Investigators of an epidemic in Philadelphia caused by several different strains of *L. monocytogenes* hypothesized that a predisposing gastrointestinal infection with another pathogen may have impaired the gastrointestinal barrier, increasing susceptibility to *Listeria* invasion.[143,144]

8.1.1. Non-perinatal Listeriosis. In immunocompromised and older adults, the clinical presentation is usually one of sepsis or meningitis, meningoencephalitis, cerebritis, or a brain abscess. The noninvasive gastrointestinal illness characterized by fever, nausea, vomiting, diarrhea, and musculoskeletal symptoms has not consistently been observed as a prodrome of an invasive infection. Focal infections, the result of or a concomitant of bacteremia, may occur at virtually any site, most commonly affecting the central nervous system. *Listeria* infection can present as cerebritis, rhombencephalitis, single, or even multiple brain abscesses.[159–162] As with *Listeria* sepsis and meningitis, these usually occur in people with complex multifaceted immune defects. In recent years, many reports of unusual localization of *Listeria* have appeared, sites including the endocardium,[163] peritoneum,[164–166] joints (usually prosthetic joints),[167–168] vascular aneurysms or grafts,[169,170] bones,[171] lymph nodes,[172] lungs, and pleura.[173–175] Respiratory failure secondary to rhombencephalitis has also been described, as has eye involvement.[176–177]

Relative to other opportunistic infections, there have been relatively few cases of listeriosis reported in patients with AIDS[12,13] This is particularly ironic since the importance of T-cell immunity and cell-mediated resistance to infection was first demonstrated experimentally with *L. monocytogenes* in the early 1960s.[152,153] That this infection is not more common in HIV infection may reflect that nonactivated macrophages, monocytes, and neutrophils may all play an important role in immune protection against *Listeria*.[154,156–158,178,179]

8.1.2. Perinatal Listeriosis. Case reports and case series have demonstrated that *L. monocytogenes* infections tend to complicate later stage pregnancies, but perinatal infection can occur as early as the first trimester.[3,123,180] Perinatal cases of listeriosis may be underdiagnosed, especially when a pregnancy ends in a fetal death. A French study cultured *L. monocytogenes* from the placentas and/or fetuses of 1.6% of all pregnancies that resulted in preterm labor or spontaneous abortion.[56] Listeriosis should be suspected in a pregnant woman with chills and fever with no obvious source, especially if the fever is accompanied by signs of a

threatened abortion. A newborn or neonate with evidence of sepsis with or without meningitis should be suspected of *Listeria* infection.

8.2. Diagnosis

Clinicians should suspect listeriosis when confronted with unexplained fever in pregnant women and immunocompromised and elderly hosts. Blood and spinal cultures, when appropriate, should routinely be obtained. Given the difficulty of isolation of *Listeria* from clinical specimens from nonsterile sites and substantial rates of asymptomatic gastrointestinal carriage, stool cultures are not sufficiently sensitive or specific to be a helpful diagnostic aid. Though not of assistance in clinical settings, improved serological tests of antilisteriolysin O may prove useful in clarifying the epidemiology of this infection. In some cases, peripheral blood monocytosis can be a clue. In cases ofmeningitis polymorphonuclear or mononuclear cell counts can range from a few toseveral thousand; in rare cases, cerebrospinal fluid cultures are positive in the absence of pleocytosis.[181] Cerebrospinal glucose concentrations range from normal to very low.

8.3. Differential Diagnosis

The differential diagnosis in a patient with meningitis includes *S. pneumoniae, Neisseria meningitidis*, or *Haemophilus influenzae*. In patients with cell-mediated immune defects, *Cryptococcus neoformans* should be included in the differential diagnosis. The absence of organisms on Gram stain may suggest tuberculosis or a partially treated bacterial meningitis as well as *Listeria* meningitis; therefore, a patient with meningitis and a CSF pleocytosis without organisms seen on Gram stain should be suspected of *Listeria* meningitis. Similarly, a clinical presentation of cerebritis with a normal CSF should also raise suspicion of *Listeria* cerebritis. In the former, the organism can be expected to be isolated from the CSF, even though not seen. In the latter, the bacteria should be isolated from blood, but may not be recovered from the CSF. The picture of clinical sepsis without meningitis or other localizing features in a patient with cell-mediated immune defects may be produced by a number of organisms, including *Salmonella* species, mycobacteria species, *C. neoformans, Histoplasma capsulatum, Coccidioides immitis*, or cytomegalovirus. *Listeria* cerebritis appears to be rather unique in that symptoms and bacteremia may occur over a longer period of time before forming a brain abscess compared with other organisms, a manifestation that has been confused with *Toxoplasma gondii* encephalitis. In the pregnant woman, the illness resembles influenza or a urinary tract infection. In the neonate, the usual causes of neonatal sepsis and meningitis such as *Escherichia coli, Klebsiella pneumoniae*, group B streptococci, the enterococci, and *Bacillus* species must be considered. There is nothing specific about *Listeria* febrile gastroenteritis, thoughconcomitant abnormalities in liver function tests may suggest the diagnosis. In compromised hosts, dual infection with *Listeria* and other microorganisms such as *C. neoformans* or *Legionella pneumophila* may occur.

9. Control and Prevention

9.1. General Concepts

Although two outbreaks have implicated pasteurized milk,[2] there is ample evidence that routine pasteurization procedures easily eliminates *Listeria*.[182,183] This stresses the importance of reducing opportunities for post-pasteurization contamination. Immunocompromised hosts, such as those with cell-mediated immune defects, and pregnant women should be educated as to the dangers of improperly processed dairy products (particularly soft cheeses), poorly washed raw vegetables, and inadequately cooked meat products. The CDC specifically recommends the following:

- Avoid foods from deli counters or thoroughly heat deli meat products before eating.
- Heat hot dogs until steaming before eating.
- Avoid cross-contaminating other foods, utensils, and food preparation surfaces with fluids from hot dog packages and wash hands after handling hot dogs.
- Thoroughly cook raw food from animal sources, such as beef, pork, or poultry.
- Keep uncooked meats separate from vegetables and from cooked foods.
- Avoid raw (unpasteurized) milk or foods made from raw milk.
- Wash hands, knives, and cutting boards after handling uncooked foods.

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis and Treatment

As person-to-person transmission is not a feature of listeriosis, isolation of patients with listeriosis is not warranted. Data on treatment for enteric listeriosis or to guide postexposure prophylaxis, especially in pregnant women, are lacking. In high-risk patients with a known exposure,

such prophylaxis is not generally recommended. Given its spectrum of activity and its ability to cross the blood–brain barrier, the antibiotic of choice has been ampicillin with or without gentamicin[159] (Table 2). Trimethoprim—sulfamethoxazole, generically called cotrimoxazole, has also been used successfully;[184] however, there are too few cases to permit a definitive judgment about its effectiveness. Comparative clinical trials have not been conducted to assess the most efficacious treatment for listeriosis.[185,186] Penicillin has been used, often with success, but concerns about relative penicillin resistance make ampicillin the beta lactam of choice; this is especially true in CNS *Listeria* infections. Although the value of adding an aminoglycoside, in particular gentamicin, has not been established by randomized prospective studies, in vitro synergism, and limited clinical data suggest the combination is indicated in severe or life threatening disease.[159] The efficacy of macrolides, vancomycin, tetracyclines, newer fluoroqinolones, carbapenems, and linezolid are uncertain, though in vitro data are encouraging for the last three.[40,159] Cephalosporins should not be used.[159]

9.3. Immunization

Because of the potential economic consequences of listeriosis outbreaks in farm animals, a variety of *Listeria* vaccines have been developed for agricultural use, but none is routinely used. No vaccine has been developed for use in humans.

10. Unresolved Problems

The CDC's active surveillance system has highlighted the importance of community-based surveillance and prompt reporting of cases. The recent descriptions of a mild form of listeriosis raise the question as to the degree to which *L. monocytogenes* is responsible for self-limited community-

Table 2. Differential Diagnosis and Treatment of *Listeria monocytogenes* Infection

		Treatment	
Category	Differential diagnosis	First choice	Second choice
Pregnancy infections	Influenza	Ampicillin	TMP-SMX, penicillin[a] (large doses), erythromycin, ? vancomycin
	Pyelonephritis Chorioamnionitis Septic abortion		
Granulomatosis infantiseptica	Neonatal sepsis	Ampicillin with or without gentamicin	TMP-SMX, penicillin[a] (large doses), erythromycin, ? vancomycin
	Neonatal meningitis due to enteric bacilli, group B streptococcus		
Sepsis	Sepsis of unknown source	Ampicillin with or without gentamicin	TMP-SMX, penicillin[a] (large doses) with or without gentamicin, ? erythromycin, vancomycin
Meningo-encephalitis	Viral encephalitis, metabolic encephalopathy or psychiatric illness	Ampicillin with or without gentamicin, possibly with gentamicin intrathecally	TMP-SMX, ? penicillin[a] (large doses) ? erythromycin and/or chloramphenicol, ? vancomycin
	Infection due to *Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, Cryptococcus neoformans*, or *Mycobacterium tuberculosis* in immunosuppressed patients or group B streptococci or enteric bacilli in neonates		
Cerebritis	Brain abscess, tumor, stroke	Ampicillin with or without gentamicin	TMP-SMX, erythromycin, ? vancomycin, ? penicillin[a] (large doses)
Focal infections	Varies with site	Ampicillin with or without gentamicin	TMP-SMX, penicillin[a] (large doses) erythromycin and/or tetracycline, ? vancomycin
Gastroenteritis	Bacterial gastroenteritis		None unless evidence of sepsis

[a]The potential for increasing penicillin resistance is an issue

acquired gastrointestinal illness. These cases could serve as sentinels for an undetected common-source food item or an outbreak.

A further investigation into the virulence factors that separate pathogenic and nonpathogenic strains of *Listeria* and more specific host factors that determine susceptibility may help to better target preventive strategies for minimizing exposure to these pathogens and further reducing the substantial morbidity and mortality that these ubiquitous organisms have on the health of the public.

11. References

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CHAPTER 23

Lyme Disease

Roger P. Clark and Linden T. Hu

1. Historical Perspective

In the short period of time since Lyme disease was first described in the mid-1970 s, it has rapidly become recognized as the most common vector-borne illness in the United States. In 1982, the Centers for Disease Control and Prevention (CDC) began surveillance of Lyme disease, and the CSTE (Council of State and Territorial Epidemiologists) classified Lyme disease as a notifiable disease in 1990.

As of 2003, every state in the United States except Montana had reported Lyme disease, with a trend toward increasing numbers of cases since reporting was first instituted. The majority of infections appear to be clustered in the Northeast from Maryland northward to Maine, the upper Midwest (Minnesota and Wisconsin), and northern California. In regions outside these areas, most, if not all, reported cases are imported. In 2005 more than 23,000 cases were reported in the United States with estimates of 6–12 unreported cases for every reported case.[13]

Lyme disease in the United States was first identified as a distinct entity in Lyme, Connecticut, where a cluster of 27 children and 8 adults had an illness manifesting with fever, myalgias, arthritis—and, most characteristically, a rash early in disease that was present in one-third of the patients.[98] This rash, which is characteristic of early Lyme disease, is known as erythema chronicum migrans, or simply erythema migrans (EM). Epidemiological studies implicated tick exposure and the treponeme-like spirochetal causative agent of Lyme disease was identified from an *Ixodes* tick and named *Borrelia burgdorferi*.[9]

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It was eventually recognized that Lyme disease in the United States had similarities to a tick-associated disease that had been recognized in Europe almost a century earlier. Afzelius described the first cases of EM lesions in European patients in 1910. Other Borrelia known to cause disease in humans have been investigated since the mid-19th century. Relapsing fever was initially proposed in 1868 to be caused by threadlike organisms by Otto Obermeier, an intern of Virchow, and acrodermatitis chronica atrophicans—a manifestation of Lyme borreliosis, was commented on by a German physician in 1883. Named successively *Spirocheta recurrentis and Spirocheta obermeieri*, these organisms finally came to be place into the genus *Borrelia* after the French scientist Borrell.[113]

2. Microbiology

Borrelia are spirochetes, belonging to family *Spirochaetaceae*.[40] Spirochetes are distinguished by a characteristic cellular structure and means of motility. *B. burgdorferi* have periplasmic flagella that are sandwiched between the outer membrane sheath and a protoplasmic cell cylinder. These periplasmic flagella, averaging from 7 to 11 in number are attached near the ends of the organism at either end and overlap at the center and account for both the shape and the motility of *B. burgdorferi*. Mutant organisms lacking the periplasmic flagella are rod-shaped bacteria and lack motility. Motility is accomplished primarily by an undulating, wavelike motion with some axial rotation, as opposed to *Leptospira* species, which achieve locomotion via a counterclockwise rotatory "corkscrew" motion.[27]

The genus *Borrelia* is composed of roughly 20 species, all of which are arthropod borne. *B. burgdorferi sensu lato* encompasses the three species that cause human disease: *B. burgdorferi sensu stricto*, *B. garinii*, and *B. afzelii*.

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B. burgdorferi sensu stricto is the only species present in the United States, while all three strains are present in Europe. *B. burgdorferi* are the longest and thinnest of the Borrelia, measuring approximately 0.2–0.3μ (micrometers) by 20–30μ (micrometers).[9,31] The genome is comprised of a linear chromosome as well as a variable but abundant number of plasmids, both circular and linear. Some borrelial isolates contain over 20 of these plasmids.[25,52] Because at least several of the plasmids are considered essential for survival of the organisms, they are sometimes referred to as "mini-chromosomes."

B. burgdorferi has proven to be a very slow growing organism, with a log-phase generation time of a half a day or more.[16,75,76] It is generally cultured on one of the variants of Kelly medium such as Barbour–Stoenner–Kelly II medium (BSK II),[6] Kelly medium Preak–Mursic (MKP),[77] or BSK-H.[76] *B. burgdorferi* grows best at temperatures between 30 and 34°C in microaerophilic conditions.

In vitro culture of *B. burgdorferi* for purposes of studying the organism and its genome has been somewhat complicated by the fact that successive generations of the spirochete grown in culture will lose substantial amounts of genetic material through plasmid shedding. Plasmids will variably and unpredictably disappear, and accompanying this plasmid loss is a concomitant loss of infectivity.[35,43,45,55,83] Specific plasmids have been associated with infectivity for *B. burgdorferi* strain B31 including LP28-1 and LP25 which are required for mammalian infection and LP25 and LP28-4 which are required for tick infection.[83,105] The critical gene on LP28-1 appears to be a nicotinamidase, *pncA*.[83]

One interesting fact about *B. burgdorferi* is that the small size of the *B. burgdorferi* genome has left it with limited to no synthetic capability for many factors normally produced by other bacteria—including amino acids, fatty acids, enzyme cofactors, or nucleotides. The result is that the organism is highly dependent upon its environment to acquire these factors and has developed a tight symbiosis with its various hosts.

3. Life Cycle

B. burgdorferi are maintained in an enzootic cycle and spread to humans only incidentally. Borrelia are harbored in the midgut of hard-bodied (*Ixodes*) ticks. In the United States *Ixodes scapularis* and *I. pacificus* are the major vectors of Lyme disease, whereas in Europe and Asia *I. ricinus* and *I. persulcatus*, respectively, are the major carriers of borreliosis. In the Southern United States, a new disease called Southern Tick-Associated Rash Illness (STARI) presenting with a rash similar to EM has been associated with the bite of the *Amblyomma americanum* tick. A different borrelial species, *Borrelia lonestari*, has been identified in Southern *Amblyomma* ticks, but the linkage of this organism to STARI is now in doubt.[40,70,110,118]

Ixodes ticks feed once during each of their three life-cycle stages (larvae, nymph, and adult). Larval forms of the ticks become infected by feeding on infected animals—most notably the white footed mouse (*Peromyscus leucopus*) which is the major reservoir for *B. burgdorferi* in the United States, although birds and other small rodents also play an important role. In Asia and in Europe birds and voles are major amplifying hosts.[25] The host preference of the tick vector is an important factor in maintenance of *B. burgdorferi* in the wild. In the Southern United States where *Ixodes* ticks feed on lizards that are resistant to *B. burgdorferi* infection and whose blood is lethal for the organism, *B. burgdorferi* is not maintained in the wild rodent population. Infected larvae pass the organism transstadially during the molt to the nymphal stage and the nymphs then infect new animals when they take their blood meal. Larval ticks are most active in the late summer; nymphal ticks in spring and early summer; and adults are most active in late summer and fall. The adult ticks will often feed on larger mammals, especially the white-tailed deer. Other large animals have been found to have high borrelial titers, including sheep, dogs, and cattle.[17,52] According to the U.S. Department of Agriculture (Agricultural Research Service), cattle may be severely affected by borrelial disease, with fever, joint swelling, weight loss, and abortion. However, infection in these large mammals is not important for the maintenance of infection in the wild because they do not serve as feeding sources for larval ticks and thus are a "dead-end" host.

Once adult female ticks have mated and fed, they will drop off into the environment where they then lay their eggs, which hatch into larvae thereby beginning a new cycle. Transovarian transmission of the spirochetes is not an important factor in maintaining the organism, as only a small percentage of eggs are infected and very few of these eggs appear to remain viable.[44,66]

4. Epidemiology

The incidence of Lyme disease has been increasing steadily since it became a reportable disease in 1990. The Council of State and Territorial Epidemiologists lists the national surveillance reporting requirements for each of the states: in 42 states, reporting is required by both the provider

and the laboratory; in the remaining eight states, the task of reporting is left to the provider only. The CDC case definition of Lyme disease for surveillance purposes is "physician diagnosed erythema migrans >5 cm in diameter or at least one objective manifestation of late Lyme disease (e.g., musculoskeletal, cardiovascular, or neurologic) with laboratory confirmation of *B. burgdorferi* infection using a two-tiered assay".[59] Nationally in 2005 there were 23,305 cases of Lyme disease reported, and the incidence of Lyme disease was 7.9 cases per 100,000 population. The incidence varies widely by state, with most cases being clustered in the Eastern coastal states from Maryland to New Hampshire plus Wisconsin and Minnesota. These states account for 95% of all cases reported in 2001 and 2002.[60] There were six states (Arkansas, Colorado, Hawaii, Mississippi, Montana, and Oklahoma) reporting no cases in 2005 (Montana had never reported a case as of 2005), while New York reported 5,565 cases.[62]

A casual glance at a bar graph showing average incidence of Lyme disease from 1992 to 2003 by age group reveals a striking bimodal distribution with the two peaks at ages 5–9 years and 50–64 years, with the lowest number of cases in the 20 to 29-year-old groups. Males had a higher incidence than females in all age groups (CDC Web site). Studies from six German states where statistics on Lyme borreliosis is monitored reflect an almost identical bimodal distribution, however, there was a slight preponderance of females (55%) affected.[56]

In addition to national surveillance data, some states also keep tally of the cases independently. Connecticut lists Lyme disease cases and rates per 100,000 population by town and country. For the year 2002 the state (CT Dept. of Public Health) reported 4,631 cases with an incidence of 136 per 100,000, with one town reporting 4995.4 cases per 100,000 (692 times the national incidence). In contrast by 2005, there was a marked decrease in the number of cases and the incidence (1,810 cases and 53.1 per 100,000 respectively). This drop was due to an unspecified change in the system of reporting cases to the state, and likely not to the actual number of cases

The state of New Jersey (NJDHSS) publishes Lyme disease rate by county. In 2000, the most recent year with both number of cases and rates published, there were 2,459 cases with a statewide rate of 29.22, with a range from 2.3 to 441.02. This was markedly increased from 1999 when there were 1,719 cases (rate of 21.11) reported. In 1989 the Georgia Department of Human Resources reported 715 cases of Lyme disease. This amounted to 11 times the total number of cases reported in the combined previous 7 years (62 cases from 1982 to 1988). This may be explained by a number of factors including the fact that free serologic testing was offered during that year, and there was an extensive educational program offered to the public and physicians in 1989. It is also suspected that there was extensive over-reporting of cases in Georgia, demonstrated by the fact that in 1989, the state used a lower threshold for detection of Lyme disease (1:128 by immunofluorescence as opposed to 1:256) than other states. In 1990 new national case surveillance definitions were released, resulting in a precipitous drop in the number of cases reported in Georgia.[58] In 2005, only six cases were reported by the CDC.

In addition to the highest clustering of Lyme disease in the northeast and northern Midwestern states, a third endemic region exists in northern California and southern Oregon. The first case in California was in Sonoma County in 1978, shortly after the initial clustering of cases in Connecticut was reported. By the year 2000, there were more than 1,700 cases (cumulative) reported in California, with 95 cases reported in 2005 (Medical Board of California, CDC DVBID). Most counties in California (52 out of 58) have reported cases of Lyme disease. The nymphal ticks have been observed to have a *Borrelia burgdorferi* infection rate of approximately 40% in parts of Mendocino county, and with the rather temperate climate that California offers, ticks have been noted to be active in all seasons of the year.

Despite some of the problems in reporting and changes in case definition, it appears that there is a true increase in the total number of cases in the United States. Some of the annual fluctuations may be due to reporting changes, variation in public and physician awareness, changes in tick habitat including weather, deer and mouse populations, and many other factors. Another potential for continued widespread distribution of both tick and pathogen is via infection of birds, which can rapidly spread the disease over a large area with their migration patterns. *B. burgdorferi* has been recovered from a variety of wild birds in North America and Europe.[2,71]

In Europe the surveillance of Lyme borreliosis differs by country, and often within countries the reporting is dependent on local notification systems. In Germany for instance, there is no nationwide reporting system, But 6 of the 16 states do have a system in place for reporting of Lyme borreliosis. Within those six states the average incidence per 100,000 population was 23.3 in 2003, which was substantially increased from the previous year when the rate was 17.8 per 100,000. The majority of these cases occurred from late spring to early fall. In neighboring Austria, the estimated incidence is 300 per 100,000. In a country of eight million inhabitants, this estimate brings the total number to around 24,000 per year. Ireland, on the other hand has an estimate 0.6 cases per 100,000, despite the widespread distribution of *Ixodes ricinus* the major vector in Europe.[115]

In Sweden, a study was performed to determine the occurrence of Lyme disease in the population of southern Sweden.[7] For the year spanning May 1, 1992 through April 30, 1993, Lyme disease was made a reportable disease. During this time there were 1,471 cases identified among a population of just over 2.1 million people. There was considerable heterogeneity of incidence by region, but the overall incidence was 69 per 100,000. The clinical presentation did not markedly differ from that in the United States.

Funding for Lyme disease research grew a significant amount from 1991 to 2000 at the National Institutes of Health (NIH), while showing a very modest increase at the Centers for Disease Control and Prevention during the same period. In fact, the single year of 1998 accounts for the entire overall growth of spending at the CDC, with a decrease in allocations in all other years. The amount allocated by the NIH grew almost 100% during this period from $13.1 million to $26 million. In the same decade, the funding for research into chronic Lyme disease increased from $124,000 to $3.5 million. This reflects the large amount of money spent for health care on the entity of chronic Lyme disease.[108]

5. Pathophysiology: Host–Pathogen Interactions

The spirochete *Borrelia burgdorferi* has evolved to be capable of flourishing in physiologic conditions that can change dramatically over a very short time span. This adaptability seems in large part due to the ability to express different outer surface proteins (Osp) under various environmental conditions. The environment found in the midgut of the tick is drastically different than in the tissues of mammals or birds—in terms of temperature, pH, and host immune responses. During a tick blood meal, Borrelia in the tick midgut experience a shift in pH from 7.4 to a more acidic 6.8. There is also a rise in the temperature as blood is ingested from the warm-blooded animal into the tick which is at ambient temperature.[99] *B. burgdorferi* expression of outer surface protein A (OspA), a protein critical for tick colonization, is increased at 23°C and decreased at 32°–37°C; the expression of OspC, a protein critical for establishment of infection in mice, follows the opposite pattern. Low pH also results in decreased production of OspA.

As expected then, OspA is expressed while the organisms are in the tick midgut, but is turned off as the blood meal is being taken. The role of OspA appears to be binding to a tick midgut protein (TROSPA), allowing the organism to colonize the tick midgut.[72] As antibodies to OspA are lethal to the *B. burgdorferi*, it is advantageous to the organism that OspA production is turned off prior to encountering the mouse immune system. As OspA is being down-regulated during the blood meal, expression of OspC is increasing[21] and OspC is expressed at high levels as the spirochetes disseminate to the salivary glands,[26] in preparation for infecting a new host. Experiments in OspC-deficient Borrelia have shown that OspC is necessary to establish infection in mice, but not to disseminate and persist once the infection is established.[28] One possible explanation for this is that OspC binds selectively to a tick salivary protein (Salp15). This protein has been shown in in vitro models to provide protection against mammalian antibodies. Accordingly, in mouse models, binding of OspC to Salp15 can enhance infectivity of *B. burgdorferi*.[85]

In addition to the effects of Salp15, tick saliva provides other advantages for the spirochetes. Tick saliva contains many factors that act as immunosuppressive agents and that suppress clotting. These actions are mediated by molecules such as histamine-binding proteins (histacalins), cytokine inhibitors (such as IL-2- and IL-8-binding proteins and interferon inhibitors), leukocyte modulators, and complement inhibitors.[69] The use of tick saliva to evade host defenses is just one example of the elegant interactions between spirochete, tick, and mammal that allows the bacteria to survive in differing adverse conditions.

Other outer surface proteins are also present and involved in the mammalian inflammatory response. Included in this group of proteins are OspE, OspF, and E/F-like leader peptide. Collectively these are referred to as OspE/F-related proteins (Erps) and they may confer some degree of protection from complement-mediated killing. This family of proteins is encoded on the cp32 plasmids, and a single spirochete may produce a variety of these Erps. They appear to be encoded solely by *Borrelia burgdorferi*, as they have not been found in other Borrelia species. Both Erps and another family of proteins, complement regulator-acquiring surface proteins (CRASPs) have been shown to help the organism evade complement-mediated killing by binding host factor H. *B. burgdorferi* has at least five separate CRASPs.[92] CRASP-1 acts by binding factor H and factor H-like protein 1 (FLH-1). These are important in regulating activity in the alternative complement pathway. On binding to factor H, complement C3b is inactivated, thereby precluding complement-mediated bactericidal effects. CRASP-1 is up-regulated during the period that a tick is ingesting its blood meal, and expression of this surface protein is linked to the environmental pH.[111] Different Erps and CRASPs are expressed by different *B. burgdorferi* species and each shows differential specificity for factor H from different hosts. It has been proposed that this may, in part, explain the species predilection for different borrelial species.

While the tick is taking a blood meal, other surface proteins are being up-regulated in preparation for host invasion. Among these are the decorin-binding proteins (Dbp) A and B. These Dbps bind to decorin—a proteoglycan that is named for its ability to "decorate" collagen fibers. Also up-regulated is P66, a porin protein which is involved in binding to activated platelets via the integrin aIIbb3 (also known as glycoprotein IIbIIIa),[11] and the fibronectin binding protein BBK032.[82] A surface protein that binds to heparin sulfate and dermatan sulfate (chondroitin sulfate B) via glycosaminoglycan adhesion is called *Borrelia* glycosaminoglycan-binding protein (Bgp).[73] Each of these proteins may help the organism attach to different host tissues.

Another potential mechanism for evading host immune responses is through antigenic variation. A 34 kDa protein named variable major protein-like sequence (Vls) remains stable until a blood meal is taken by the tick. This triggers a genetic shuffling of DNA cassettes on the plasmid lp28-1. The resultant heterogeneous population of Vls variant spirochetes likely contributes to immune evasion, as suggested by the fact that loss of the Vls containing plasmid results in decreased infectivity.[43]

As organisms that disseminate in tick and mouse hosts, Borrelia require a mechanism for breaking down extracellular matrix barriers. Because *B. burgdorferi* do not produce exported proteases capable of digesting extracellular matrix proteins, it must acquire this functionality from its hosts. *B. burgdorferi* has been shown to bind plasmin and plasminogen activators such as urokinase. Binding of these proteases to *B. burgdorferi* stabilizes them and protects them from inactivation.[12,23,33,41] Binding of plasmin, which in addition to being a component of the fibrinolytic system is a broad spectrum protease, aids the organism in digesting extracellular matrix tissues and moving from the tick midgut to salivary gland and possibly in disseminating from the tick insertion site to infect distant organs. The mechanism of action of plasmin is likely enhanced by its ability to activate host matrix metalloproteinases (MMPs), which are enzymes specifically designed to digest extracellular matrix that are induced from host cells by *B. burgdorferi*. *B. burgdorferi* induces production of MMPs from multiple different tissues and the presence of active MMPs is thought to be a major factor in the development of Lyme arthritis.[32]

6. Spectrum of Disease/Clinical Manifestations

Lyme disease is an illness that can range from asymptomatic (about 10% of *B. burgdorferi sensu stricto* and probably a higher percentage of *B. afzelii* and *B. garinii* infections lack symptoms) to severe, involving many organ systems. The manifestations are typically divided into early- and late-stage symptoms. The earliest consequence of *B. burgdorferi* infection is a distinctive, transient, expanding circular or ovoid macular rash called erythema migrans (EM) that is noted at the site of the tick bite in 70–90% of cases.[94] The rash is often described as a "bull's-eye" where there is a central clearing surrounded by an expanding lesion; however, this is seen only in a minority of EM rashes. It generally evolves at the site of the bite within 5 days to 2 weeks.[67] This rash is usually non-painful, and non-pruritic, beginning as a small macular or papular lesion and expanding over a period of days to several weeks. As awareness of this condition becomes more universal in endemic areas, recollection of this erythema migrans rash has been enhanced and later studies show a much higher prevalence than initial reports indicated.

Most patients with EM rash also experience other systemic complaints concurrent with the lesions. These symptoms, which are non-specific and generally transient, include fever, headache, malaise, and myalgias. Patients may experience lymphadenopathy of nodes draining the affected site. Other early manifestations include arthralgias (distinct from true arthritis that manifests later in the course), cardiac, and neurologic findings as detailed below.[60]

6.1. Cutaneous

The localized EM rash is often followed days to weeks later by the development of multiple disseminated EM lesions. In addition, patients may develop borrelial lymphocytomas—dense lymphohistiocytic infiltrations resembling dermal lymphomas.[4] This seldom if ever found in the United States. Another clinical manifestation of Lyme borreliosis found almost exclusively in Europe is acrodermatitis chronica atrophicans (ACA), a sign of late infection involving primarily the acral regions. It is more common in the elderly and may persist for years. It is characterized by diffuse spreading inflammation of the upper dermis, leading to central skin atrophy and hyperpigmentation. These areas usually become devoid of hair and may exhibit marked irritation such as itching or hyperaesthenia. These traits may resolve to some degree with the administration of antibiotics. Borrelia have been cultured from an ACA lesion that had persisted for at least 10 years, demonstrating the ability of these organisms to thrive in the host for a remarkably long period of time.[3,5] Although the lesions tend to occur in cooler sites such as distal limbs, they have been noted to occur anywhere on the body, and not necessarily in the region of the initial EM rash.

Recently, *Borrelia burgdorferi* has been implicated as possibly being associated with mycosis fungoides, a cutaneous T-cell lymphoma. In skin biopsies taken from 83 patients with mycosis fungoides, *B. burgdorferi* was detected by PCR in 15 cases and in 0 out of 83 matched controls ($p < 0.0001$). It is hypothesized that *B. burgdorferi* may stimulate a protracted Th-1 response with accompanying build-up of CD4+ T cells, possibly leading to malignant transformation;[106] however, these studies require further confirmation.

6.2. Cardiac

Cardiac manifestations are less common than skin symptoms. The most common of the cardiac manifestations of Lyme is heart block that can vary from first degree to complete heart block, usually with a duration of under a week.[54,95] In a CDC report of 31,120 patients in 2001–2002 in the United States <1% had heart block. Studies in Europe reveal the same relative infrequency of this complication.[46] Myopericarditis occurs even less frequently, and ventricular dysfunction or failure is rare. High degree heart block may require the placement of a temporary pacemaker, but is usually transient with or without treatment with antibiotics. Cardiac complications occur during the early phase of disease and are generally not seen in late-stage Lyme disease.

6.3. Neurologic

All three species contained in the *Borrelia burgdorferi sensu lato* group have significant tropism for neurologic tissue. Of these organisms, *B. afzelii* is least likely to cause nervous system manifestations. *B. burgdorferi sensu stricto* and *B. garinii* can both present with a sometimes bewildering range of nervous system features that can prove to be confusing when no rash was initially identified.

Neurologic disorders may present with either peripheral nervous system (PNS) or central nervous system (CNS) involvement, with PNS symptoms being much more commonly reported.[29] In Europe, meningoradiculoneuritis with lymphocyte pleocytosis in the CSF (Bannwarth's syndrome) is a commonly seen neurologic phenomenon. Peripheral nerve palsies can affect many different nerves with facial nerve involvement being common. Lyme-related facial nerve palsies may at times present bilaterally—which is rare for other causes of facial palsy, but occurs in up to 10% of patients with Lyme facial palsy. Other facial nerves may be affected, including cranial nerves III–VI and VIII, which can result in balance and hearing deficits. Other forms of mononeuropathy multiplex include involvement of the brachial or sacral plexus, radiculoneuropathies (often described as "patchy and painful"), motor neuropathies,[29] and even optic neuropathy, a rare disorder found mostly in children.[86] The optic neuropathy may result in irreversible blindness even with optimal antibiotic treatment.

Other early neurologic manifestations may include disorders of cerebellar function, including ataxia, myelitis, and encephalitis. More commonly noted is Lyme meningitis which manifests as headache and neck stiffness due to the presence of the spirochetes in the CSF. Differentiating meningitis caused by this organism from other types of "aseptic" meningitis may be clinically difficult at times, as most (entero)viral meningitis occurs with a seasonality that parallels that of Lyme infection. Distinguishing features beside EM rash include the presence of cranial neuropathy and papilledema. Nearly 90% of those with Lyme as an etiology exhibit at least one of these features, compared to none of the patients with enteroviral meningitis.[19,89]

Neuroborreliosis can persist into late-stage disease causing a wide array of complications. Mild encephalopathy with decreased cognitive ability is the most common complaint. In those patients who have never received antibiotic treatment, this impairment may normalize with standard therapy. Polyneuropathies and radiculitis that developed during acute disease may persist into late-stage Lyme disease. Some of the symptoms of neuroborreliosis may linger for many years, although the majority of patients have complete resolution after weeks to months. In a Scandinavian study[7], a five-year follow-up survey of patients with documented Lyme neuroborreliosis showed that 75% of patients had a complete recovery, with 61% within the first 6 months. Those with residual problems reported difficulty with concentration, persistent facial nerve palsies, neuropathies, and/or paresthesias.

6.4. Musculoskeletal

Arthritis was the hallmark clinical finding that led to the recognition of Lyme disease in the United States. It is now known that roughly one half of those infected with *B. burgdorferi* will develop arthritis during the later stages of disease if not treated with antibiotics.[96] In Europe, there may be a lower incidence, especially in those infected by *B. afzelii*. Lyme arthritis is characterized by a relapsing and remitting form of mono or oligoarthritis that usually affects the larger joints, with the knee joint being involved most often. Of note, differentiating Lyme arthritis from other common causes of arthritis, the temporomandibular joint (TMJ) is sometimes affected.[96] Pathologic specimens of synovial tissue from patients with Lyme arthritis demonstrate mononuclear infiltration, proliferation of vasculature

as well as synovial thickening or hypertrophy. The frequency of arthritic episodes usually becomes more spaced out until eventually there is complete resolution even in the absence of antibiotic therapy. Seldom do arthritic flares occur beyond 5 years. In those patients who are treated early on in the course of Lyme disease, arthritis seldom occurs.[15] In those who are initially untreated, and develop arthritis, antibiotics will generally shorten the duration of symptoms.

A minority of patients treated with antibiotics will continue to have a more continuous, persistent arthritis that has been designated by some researchers as "antibiotic-resistant chronic Lyme arthritis." The exact mechanism of disease in these patients remains somewhat controversial, with theories centered around two core ideas: 1) an autoimmune process resulting from molecular mimicry or 2) persistence of *B. burgdorferi* (or borrelial antigens) in the joint space. *B. burgdorferi* DNA has been identified by PCR in the synovial membrane of some patients who have had extensive antibiotic therapy for Lyme arthritis.[81] Other studies have not found borrelial DNA present in large numbers of synovial fluids from patients with persistent disease. While in vitro antibiotic resistance to commonly used antibiotics has not been noted, it does not rule out the possibility of in vivo resistance of *B. burgdorferi* in association with tissues. As we will discuss in the Treatment section, there is growing evidence for persistence of *B. burgdorferi* after antibiotic therapy in both animal models and humans with Lyme disease.

There has also been some evidence to support an autoimmune mechanism for arthritis in antibiotic-resistant patients. Patients with treatment-resistant Lyme arthritis have an increased incidence of MHC class II haplotypes HLA-DRB1*0401 and 0101.[36] These haplotypes have been associated with rheumatoid arthritis and therefore serves as an attractive candidate to function as a mediator of autoimmune arthritis. Antibodies to OspA are found at greater frequency in patients with antibiotic-resistant Lyme arthritis,[37] and it has been suggested that molecular mimicry between an epitope of OspA and the human leukocyte function-associated antigen-1 (LFA-1) may be the mechanism for T-cell cross-reactivity. Subsequent data has not supported LFA-1 mimicry as a mechanism for antibiotic-resistant arthritis;[38] however, this does not rule out the possibility that a different molecular mimic exists.

The entity of "chronic Lyme disease" receives much attention in the lay press and remains a controversial topic. A multitude of unexplained illnesses, arthralgias, fatigue, and depression are all attributed to chronic Lyme disease, usually without any objective evidence. Some clinicians have even diagnosed "sero-negative" Lyme disease in patients with these non-specific symptoms and negative laboratory testing for Lyme disease. Several large studies have not shown an increase in these non-specific symptoms after infection with *B. burgdorferi.*

7. Possible New Borrelial Syndromes

In the southern United States, where Lyme disease is not endemic, a tick-related erythema migrans-like rash has been recognized.[40] The tick associated with this entity, known as southern tick-associated rash illness (STARI), is *Amblyomma americanum* or the Lone Star tick. It is generally described as a very mild illness, usually consisting of rash without systemic manifestations such as fever or the multitude of complaints described in Lyme disease. Currently, as STARI is defined by the rash it produces. It has been found to be a distinct illness from Lyme disease as patients are test negative for Lyme disease by all accepted methods. In a comparison between patients with EM rash in Missouri and New York, the appearance of rash was earlier in patients from Missouri; they were less likely to have multiple lesions and the lesions were generally smaller and more likely to have central clearing.[118] *Borrelia burgdorferi sensu stricto* is not found in the tissues of those affected by culture or PCR. Another borrelia, *B. lonestari* has been suggested as a possible cause of STARI. *A. americanum* ticks in the southern and south central United States have been found to contain *B. lonestari* DNA.[10] In a single instance, a tick identified as *A. americanum* was detached from the center of an erythema migrans rash in a man who lived in New York and had recently traveled to Maryland and North Carolina. Both the tick and a biopsy from the rash contained DNA from *B. lonestari.* No evidence of *B. burgdorferi* was found from either source. However, a recent study in Missouri, 30 patients with EM rash had skin biopsies performed, none of which yielded DNA for either *B. lonestari* or *B. burgdorferi*.[117] The cause of STARI should be considered unknown at the present time.

Recently in Europe there was a case report of a more severe illness caused by a Borrelia previously not known to be pathogenic. *Borrelia lusitaniae* has been found in *Ixodes* ticks in coastal North Africa and Portugal and is implicated in a chronic rash with associated symptoms (headache, cardiac rhythm disturbances, and paresthesias) in a Portuguese woman. The organism was cultured from a skin lesion.[115]

8. Diagnosis

Early Lyme disease with a classic EM rash in a person with tick exposure in an endemic area is strictly a clinical diagnosis and requires no laboratory testing.

There are several methods for directly or indirectly detecting the presence of Borrelia from tissues or fluids. Cultures and PCR directly detect the presence of *B. burgdorferi* or its DNA. However, due to the difficulties with using these tests in a clinical setting, the vast majority of cases are diagnosed by serologic testing. There are a great number of laboratory tests available to the clinician and laboratory technologist, so great care must be taken to order and interpret these tests appropriately. The FDA has given approval to over 70 serologic assays alone.[61] Unfortunately inter-test reliability and even intra-test reliability in different laboratories remain a significant issue.

Most laboratories rely on enzyme-linked immunosorbent assay (ELISA) testing as the initial test. Depending upon the cutoff values employed, its sensitivity approaches 90%, with a specificity of 72%.[107] A negative test, especially in a setting with a low pretest probability makes the diagnosis of Lyme disease very unlikely. ELISA testing can have false-positive results due to cross-reactivity to other spirochetes such as *Treponema pallidum* or *Leptospira interrogans*. Patients with prior exposure to other borrelial illnesses such as relapsing fever may also test positive. In addition, autoimmune diseases such as Lupus may trigger a false-positive result with ELISA. With indeterminate or positive ELISA results, a follow-up Western blot is generally performed in order to rule out these false-positive results. The Western blot detects specific proteins or peptide segments rather than intact organisms and has a higher specificity than does standard ELISA. Both ELISAs and Western blots can be performed for IgM (early) and IgG (late) antibodies. Current recommendations suggest a cutoff of two out of three antigens positive for IgM testing and five out of ten antigens for IgG testing.[18] Of note, in patients with untreated disease for longer than 1 month, a positive IgM alone in the absence of a positive IgG test should be interpreted as a negative test. IgM testing is more frequently prone to result in false-positive testing.

Newer generation ELISA tests using synthesized or recombinant antigens such as the C6 peptide antibody assay are showing some promise in improving sensitivity and specificity of detection. The C6 ELISA tests for levels of IgG against a conserved region of the VlsE protein. This peptide evokes a positive IgG response as early in disease as IgM in traditional ELISAs and likely has fewer false positives.[51] Additionally, the C6 ELISA can be used in those patients who have previously been vaccinated with the new withdrawn OspA vaccine.[51] The epitope is conserved in *B. garinii* and *B. afzelii*, so it may be an appropriate test for patients in Europe where testing is complicated by the presence of multiple strains with differing reactivity to standard ELISA tests. While some studies show very good sensitivity and specificity,[63] the use of the C6 assay alone without confirmatory testing is not yet considered standard practice.

Great care must be taken when considering whether to send samples for serologic testing. It has been calculated that if the pretest probability of Lyme disease is <0.2, then laboratory testing is unlikely to provide useful results and in fact is more likely to be a false positive than true positive.[107] Therefore, the clinician should have a solid clinical suspicion (e.g., unexplained oligoarthritis and exposure to an endemic area, etc) before ordering these studies. In highly endemic areas, the absence of EM rash cannot be used to completely rule out Lyme disease however, and serologic testing can be helpful diagnosing conditions felt to be due to Lyme disease.

9. Special Considerations

9.1. Pregnancy

Congenital Lyme disease appears to be a rare phenomenon. In 1986, researchers [50] reported 19 cases of maternal infection by *B. burgdorferi* between 1976 and 1984. Thirteen of these women were given antibiotic treatment and five of the pregnancies resulted in adverse outcomes, including cortical blindness, syndactyly, and intrauterine death. In none of these cases was the cause directly attributed to the spirochete, but the high frequency of adverse events was noted. There have been reports of transplacental passage of *B. burgdorferi* to the fetus, and in at least one case, this occurred despite maternal treatment with oral penicillin.[113] One such case resulted in a congenital cardiac defect,[87] although again the cardiac defect could not be directly attributed to the intrauterine infection.

Several large studies have failed to conclusively show an association between maternal infection and adverse neonatal outcomes.[24,47,64,104] In the study by Nadal et al., of 12 women who were seropositive, the single mother with clinical Lyme disease had a neonate with a ventricular septal defect. Silver[91] provides a review of the studies and concludes that Lyme disease in pregnancy has a rather benign course.

Current recommendations are to treat infected pregnant women with the same treatment that would be recommend if the patient were not pregnant with the exception that doxycycline should be avoided. In a very small prospective study, breast feeding by symptomatic mothers with Lyme disease did not result in any adverse outcomes.[120]

9.2. Immunosuppression

There are currently no reports to suggest that immunosuppressed patients (HIV, chemotherapy, transplantation,

splenectomy) have more severe courses of Lyme disease than immunocompetent patients or have a lower rate of response to antibiotics.

9.3. Co-infection

Ixodes ticks may carry other bacteria or viruses that may be transmitted to humans along with *B. burgdorferi*. Ticks in the northeastern and Midwestern United States are frequently co-infected with *Babesia microti* and/or *Anaplasma phagocytophilum*. In one study, for instance, in New Jersey, DNA analysis of ticks showed *B. burgdorferi* and *Babesia microti* co-infection in 1.9% of ticks harvested.[1] Similar rates were found in the upper Midwest by analyzing serologic evidence of co-infection in humans.[57] In contrast, in the highly endemic areas of Connecticut and Rhode Island, 11% of subjects had serologic evidence of Lyme disease and *Babesia* co-infection.[42] There are limited studies that suggest that infection with more than one of these organisms may result in a more severe course for Lyme disease; however, the differences, if they exist, are typically small. Tick-borne encephalitis is a viral disease carried by *Ixodes* ticks in Europe and Asia and can cause severe neurologic problems. Interactions with *B. burgdorferi* infection have not been reported.

10. Prevention

Because Lyme disease is spread by ticks, the simplest preventive measure is tick avoidance. One risk factor for exposure to ticks is yard size. Yards larger than ½ acre are more likely to be wooded and to supply habitat for ticks and their mammalian hosts.[53] People who spend 30 hours per week or more engaged in leisurely outdoor activities are significantly more likely to become seropositive for Lyme disease than controls. The use of clothing to prevent ticks having access to skin is recommended. Long-sleeved shirts and long pants tucked into socks may be beneficial. The use of light-colored clothing makes ticks more visible and easier to remove and to serve as a warning of local tick infestation. The use of whole body checks after exposure to areas where ticks are felt to be present and during warm weather is advised, as transmission of *B. burgdorferi* is felt to be low in the first 24–48 hours of attachment.

Also advisable as part of personal protection against tick bites is the use of insect repellants. DEET (N,N-dimethyl metaloluamide)-containing formulations have been the standard repellant in the United States for many years. It is available in various concentrations from 5% up to 100%, with higher concentrations having more prolonged protection. It has been shown to be a safe and effective repellant against mosquitoes, ticks, chiggers, and biting flies. In Europe and Australia, the repellant Picaridin (KBR 3023) has been in use for many years and is also a safe and effective mosquito repellant.[109] Less information is available about its effectiveness against ticks. In a South African study[78] Picaridin was found to have limited effectiveness against *Amblyomma hebraeum* (the vector in African tick bite fever). Permethrin, which is not a repellant, but rather an insecticide (acaricide) is perhaps the most effective means of preventing tick bites.[20] Permethrin is to be applied only to clothing and not to skin.

The population of ticks in residential areas can be curtailed by simple landscaping procedures such as creation of a buffer zone of mulch between the grassy yard and the abutting areas of woodland and scrub, where ticks reside. The removal of branches and leaves and once or twice yearly application of acaricides can significantly reduce exposure to ticks. Application of carbaryl (an acaricide) to residential yards can be nearly 100% effective against *Ixodes* ticks, with residual cidal effects of up to 60 days.[93] Another acaricide, deltamethrin has been demonstrated to reduce both *Ixodes dammini* and *Amblyomma americanum* ticks when applied to the wooded periphery of residential sites.[88]

Another approach to controlling the tick vector of Lyme disease is the application of acaricide to the mammalian hosts, such as the white-tailed deer. By placing feeding stations in strategic locations, deer in the wild are attracted to food such as whole kernel corn. In order to access the feeding bin, the deer must rub up against foam rollers that have been impregnated with acaricide.[74]

In 1998 a human vaccine against *B. burgdorferi* was licensed. Named LYMErix, this vaccine consisted of a recombinant OspA lipoprotein. This vaccine was unique in that it acted by a human antibody-mediated effect against *B. burgdorferi* that took place in the tick vector rather than in the human host. OspA is up-regulated in the tick and is generally not expressed in mammals. After a series of three injections, the efficacy against Lyme disease was 76%. It was also established to be a safe vaccine, with the chief side effects consisting of local injection site reaction, myalgias, and a small number of cases having fever (2%).[39,97] Despite the attributes of good efficacy and safety, the vaccination was withdrawn from the market in 2002 by the manufacturer due to small demand. This low demand was partly the result of claims that the LYMErix vaccine could theoretically cause autoimmune arthritis through molecular mimicry of LFA-1 by OspA. Large studies have never supported an association of the vaccine with arthritis. Recently, trials of a new OspA vaccine which has been engineered to remove the LFA-1 cross-reacting epitope have started in Europe.

The use of antibiotic prophylaxis in a person found to have and attached tick is somewhat controversial. One study performed in New York state in an area known to have very high rates of Lyme disease showed that treatment with single oral dose of doxycycline was efficacious in preventing the development of Lyme disease.[65] Another slightly smaller study in southeastern Connecticut failed to show any difference in risk of infection of children given amoxicillin prophylaxis.[90] The routine use of antimicrobial prophylaxis is not recommended by the Infectious Disease Society of America (IDSA).[119] In those patients who discover an attached tick, careful observation is advised for signs of Lyme disease, or other tick-borne illnesses such as babesiosis or anaplasmosis. The use of prophylactic antibiotics should be restricted to patients in endemic areas who present with a *Ixodes* tick bite where the tick has been attached for greater than 36 hours.

11. Treatment

Treatment of Lyme disease is dependent on the clinical manifestations present. The following treatments are extracted from the Infectious Diseases Society of America (IDSA) practice guidelines for the treatment of Lyme Disease.[116] In patients with known or suspected tick bites who manifest with signs and symptoms of Lyme disease, especially with a rash consistent with erythema migrans, oral treatment should be initiated. Doxycycline 100 mg twice daily (in patients >8 years of age) for 14 days (range of 10–21 days) or amoxicillin 500 mg three times daily for 14–21 days have been recommended as first-line therapy by expert panels. The use of doxycycline is preferred in adults as it is also effective against *Anaplasma phagocytophilum* and has good penetration into the CNS. Cefuroxime (500 mg twice daily) for 14–21 days has also been shown to be very effective. Doxycycline should not be taken by pregnant or breast-feeding women, or children less than 8 years old. Newer macrolide antibiotics are also effective, but the treatment failure rate is higher than for first-line regimens.

For Lyme arthritis, oral or intravenous antibiotics may be used. Oral doxycycline is again the preferred drug, as it is well absorbed, inexpensive, and efficacious. It also has some inherent matrix metalloproteinase inhibitory properties, thus acts as an anti-inflammatory agent. Amoxicillin or cefuroxime is alternative agent as noted above. The recommended length of treatment is a 28-day course. Some experts advocate an additional 28-day course of PO or IV antibiotics when patients have not responded to the initial course. PCR testing of the joint fluid may be helpful in confirming clearance of the organism and guiding length of therapy. Patients with negative PCR testing who have not improved with antibiotic therapy often respond to traditional treatment for rheumatoid arthritis. Anecdotal responses to methotrexate and anti-TNF therapies have been described.

Intravenous therapy is often recommended for patients with neurological or cardiac complications of Lyme disease. The exceptions are those with neurological disease limited to facial palsy without meningitis and cardiac disease limited to first or second degree AV block. These patients may be treated with the oral regimens described for EM. Some experts recommend routine lumbar puncture for all patients with facial nerve palsy due to Lyme disease, while others reserve lumbar puncture for those with headache, neck stiffness, or other signs of CNS involvement. For patients with inflammatory changes in the CSF, intravenous penicillin (18–24 million units iv daily, divided q 4 h), cefotaxime (2 g iv q 8 h), or the preferred regimen of ceftriaxone (2 g once daily) should be initiated. There have been no trials aimed at the most appropriate treatment for Lyme carditis, the current guidelines are based on expert opinion, but have been established as standard of care. Hospitalization and cardiac monitoring are recommended for symptomatic patients with atrioventricular heart block or Lyme myocarditis. Initial therapy generally should be intravenous β-lactam antibiotics such as ceftriaxone. Once the heart block is considered stable, and if temporary pacemaker is not required, the treatment can be transitioned to oral antibiotics for a total course of 14–21 days.

For those patients with acrodermatitis chronica atrophicans, data suggest that a 21-day course of the oral antibiotics used for erythema migrans is generally sufficient. No controlled trials have been conducted comparing oral to intravenous therapy for this condition.

While the organisms are sensitive to killing by multiple antibiotics and the development of resistance to antibiotics approved for treatment of Lyme disease has not been documented, there is now growing evidence that *B. burgdorferi* may persist in animals following administration of antibiotics. Straubinger et al. infected dogs with *B. burgdorferi* and treated with different regimens of amoxicillin, doxycycline, azithromycin, or intravenous ceftriaxone.[100,101,102] Although skin punch biopsy culture rapidly turned negative in animals within 2 weeks of initiation of antibiotics, 17% of the antibiotic-treated animals were culture positive for *B. burgdorferi* on post-mortem tissue cultures >3 months after antibiotic therapy. In addition, at least one PCR of a skin punch biopsy or post-mortem tissue was positive for all but one animal (11/12 total—six treated with doxycycline and six with amoxicillin) beyond 80 days after therapy. Studies have shown that positive testing for *B. burgdorferi* DNA by PCR correlates well with persistence of live organisms,[49]

however, this may not be true in all cases and PCR has the potential to recognize DNA from dead or dying bacteria. Antibiotic-treated dogs had fewer symptoms than control-treated dogs although several had mild arthritis. Interestingly, antibody titers declined in the antibiotic-treated dogs despite the continued presence of bacteria as detected by culture or PCR, suggesting that a minimal threshold of organisms is required to maintain antibody titers or that the bacteria that remain after antibiotic therapy express antigens not detected by the ELISA assay used.

Recently Bockenstedt et al. and Hodzic et al. reported on the persistence of *B. burgdorferi* after antibiotic treatment in mice.[8,30] Dr. Bockenstedt's group administered either ceftriaxone or doxycycline to mice 30 days after infection. Mice were treated for 30 days with antibiotics. At 1 month and 3 months after treatment, the presence of *B. burgdorferi* was tested by xenodiagnostic feeding of uninfected nymphal *Ixodes* ticks on the mice. About 40% of mice in the both the ceftriaxone- and the doxycycline-treated groups tested positive by xenodiagnosis in that spirochetes could be observed in ticks by direct fluorescent antibody microscopy (DFA) or their DNA detected by PCR. Dr. Stephen Barthold's group performed similar experiments but also compared the effects of early (within 3 weeks of infection) and late (4 months after infection) antibiotic therapy.[30] With the initiation of early therapy, none of the antibiotic-treated mice were positive on culture or PCR of tissues. However, one of three mice was positive by xenodiagnosis. In mice treated late after infection, and evaluated 1 month after treatment, tissues from all of the antibiotic-treated mice remained PCR positive (but culture negative) and 37–60% of mice were positive by xenodiagnosis suggesting that the timing of antibiotic therapy plays an important role in antibiotic-mediated eradication of Borrelia. At 3 months after infection, tissues from two of five antibiotic-treated mice were positive for *B. burgdorferi* by PCR and 40% were positive by xenodiagnosis. Quantitative PCR revealed that spirochete numbers in the mice with persistent infection after antibiotics were significantly lower. Similar to the dog experiments, serum antibodies against *B. burgdorferi* also decreased in the antibiotic-treated compared with saline-treated mice despite the continued presence of the organism. When ticks that fed on antibiotic-treated mice were used to subsequently feed on immunodeficient mice (to increase the chance of establishing infection), 89% of the mice showed evidence of acquiring *B. burgdorferi* by PCR.

Data for persistence of *B. burgdorferi* after antibiotic therapy in humans are less clear. There have been multiple reports of relapses/failures of antibiotic therapy in patients with Lyme disease; however, some of these were not culture proven or were using antibiotics not currently recommended for the treatment of Lyme disease.[68,79,80,103] Certain antibiotics do seem to be less effective clinically (e.g., erythromycin) despite in vitro activity.[114] PCR from synovial tissue of patients with Lyme arthritis have been reported to remain positive after antibiotic therapy.[81] PCR from synovial fluid after antibiotic therapy, which could detect either live or dead bacteria, can remain positive after 1 month of antibiotic therapy, but is generally cleared after 1–2 more months of antibiotics. Cultures of synovial fluid are uniformly negative both before and after antibiotics, likely due to the limitations of culture. Strle et al. have reported that 31% of patients with a positive culture from an EM lesion remain positive 3 months after a 14-day course of doxycycline; similarly 6% of patients treated with azithromycin remained positive by culture.[103] Hunfeld et al. have reported that skin biopsies of patients with culture positive EM remained *B. burgdorferi* positive by culture 2 months (range 1.3–3 months) after antibiotic therapy in 1.7% of patients (19 out of 1148).[34] Spirochete persistence was not accounted for by the development of resistance to the antibiotic. However, there have been concerns raised about this study, because the strains of *B. burgdorferi* recovered in some cases differed from those found in the EM lesion prior to antibiotic therapy. Whether persistence of *B. burgdorferi* after antibiotic treatment could be a cause for persistent symptoms in some patients remains unknown.

12. Conclusion

While research over the last several decades has greatly increased our knowledge about Lyme disease, progress is still needed in the areas of reliable diagnosis and prevention. Recent advances in the understanding of how *B. burgdorferi* interacts with its various hosts may lead to new concepts in testing and treatment of human disease and control of infection in the wild-life reservoirs.

13. References

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CHAPTER 24

Meningococcal Infections

Robert S. Baltimore

1. Introduction

The meningococcal diseases[1] represent a spectrum of illness caused by *Neisseria meningitidis* (Table 1). Although sporadic endemic cases occur throughout the world, massive, devastating epidemics tend to reflect conditions of crowding, mobilization, and enclosed institutional populations. In recent years outbreaks have also been associated with large religious gatherings, especially the Hajj. Such outbreaks tend to be extraordinarily disruptive, especially because of the fear and fright that they induce in the affected populations. Among civilians, children are most often attacked, with mortality rates of 80–90% having been noted in some epidemics that occurred before effective therapeutic agents became available. In the past the disease was also known as "cerebral spinal fever" and "epidemic cerebral spinal meningitis," as well as by other names. Mobilization for war, with the induction of many young men into crowded military camps, has generally been accompanied by outbreaks. Also, in recent years there has been awareness of outbreaks as well as frequent sporadic meningococcal infections among college freshmen, who also represent a mix from many diverse origins, in dormitories and other sometimes crowded living quarters on many campuses. Generalized outbreaks among the military do not arise de novo, but may reflect what is occurring in the associated civilian populations. The development of meningococcal capsular polysaccharide vaccines, and more recently conjugate meningococcal vaccines, has had a significant effect on prevention of military epidemics of meningococcal epidemics, and now its impact on civilian disease is, becoming, realized.

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In the first edition of this book, this chapter was written by the late Harry Feldman, MD. Substantial historical and epidemiological data come from his original manuscript.

[1] In this chapter, the term *case* or *disease* refers to any instance of meningococcal infection other than asymptomatic pharyngeal (carrier).

2. Historical Background

Clinical meningococcal disease was first described by Vieusseux[(1)] in 1805 during an outbreak in the vicinity of Geneva, Switzerland. A year later, Danielson and Mann[(2)] reported a small, probable epidemic of meningococcal meningitis in Medfield, Massachusetts. This paper appeared in a publication known as the *Medical and Agricultural Register* and was entitled "The history of a singular and very mortal disease, which lately made its appearance in Medfield." The nine fatal cases were so similar that only one was fully described; all occurred during March. Weichselbaum[(3)] identified meningococci in the spinal fluids of patients in 1887.

During the 19th century, possible epidemics occurred in countries throughout the world in 1805, 1837, 1854, 1876, and 1896. In the 20th century, major outbreaks were noted during World Wars I and II, and subsequent to the latter, there was a massive, lengthy outbreak in sub-Sahara Africa,[(4)] a more localized one in Morocco,[(5)] and another affecting more than a quarter of a million people in Brazil,[(6)] as well as others in Finland[(7)] and among American military inductees during the Korean and Vietnam mobilizations. Smaller, localized outbreaks have occurred from time to time in various institutions and localities. In the course of the many studies that were conducted during World War I, Gordon[(8)] identified four serological types of meningococci: I, II, III, and IV. In 1953, Branham,[(9)] as the result of her careful, meticulous studies, labeled the different serogroups A, B, C, and D. She believed that organisms of group A were those principally responsible for epidemics and that sporadic cases

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Table 1. Clinical Spectrum of Symptomatic Meningococcal Infections

Meningitis
With meningococcemia
Without meningococcemia
Septicemia
Purpura
Adrenal hemorrhage
Chronic meningococcemia
Recurrent meningococcemia with complement deficiency
Occult bacteremia
Pneumonia
Other focal infections
Conjunctivitis
Endophthalmitis
Septic emboli to skin
Pericarditis
Myocarditis
Urethritis
Arthritis
Epididymitis

tend to be antigenically heterogeneous as the result of infections with groups B and C. Slaterus[10] subsequently demonstrated three additional meningococcal serogroups (X, Y, and Z), and then Evans et al.[11] identified serogroups W-135 and 29-E from US Army isolates. Subsequently, groups H, I, K, and L have been described[12,13] In the 1970s and 1980s, groups Y and W-135 became recognized as frequent causes of invasive meningococcal infections.[14–17]

An enormous step forward was made early in World War II when it was learned not only that sulfadiazine was effective in the treatment of cases but also that small doses eliminated the organism from the nasopharynx.[18–21] When sulfadiazine was administered on a mass basis to a closed population, cases of disease disappeared almost instantaneously and the carrier rate was reduced to near-zero levels. Thus, an effective, simple, relatively inexpensive control system for preventing meningococcal disease and curtailing epidemics was provided. Unfortunately, this was found to be ineffective in 1963 by Millar et al.[22] during several unsuccessful attempts to control the disease among naval recruits in San Diego. It was learned then that many disease-related strains were resistant to large concentrations of sulfadiazine, and thus that the sulfonamides could no longer be used effectively for the prevention or control of epidemics.[23] In a number of subsequent trials conducted under the auspices of the Armed Forces Epidemiological Board, no substitute chemotherapeutic agent was found, although the satisfactory treatment of actual cases could be accomplished with large doses of penicillin. The latter was quickly adopted as routine therapy for both military and civilian use. Eventually, in the 1970s, rifampin was adopted as the recommended chemoprophylactic agent for close contacts of cases,[24] and currently a number of agents are useful for prophylaxis (Table 2).

In the early 1970s, a vaccine for serogroups A and C was developed.[25,26] In 1981 a tetravalent vaccine (Menomune®) containing the capsular polysaccharides of serogroups A, C, Y, and W-135 was marketed commercially and is available today for the military and individuals considered to be at exceptional risk of contracting meningococcal infection.

In the late 1990s there was considerable concern about the risk of meningococcal disease in freshman college students. This led to strong recommendations for routine immunization of this group with meningococcal polysaccharide vaccine.

In January 2005 a tetravalent meningococcal conjugate vaccine (MCV4) containing the capsular polysaccharides from serogroups A, C, Y, and W-135 (4 μg each) conjugated to 48 μg of diphtheria toxoid was approved in the United States for immunizing people 11–55 years

Table 2. Chemoprophylaxis for Contacts of Patients with Infection Due to *Neisseria meningitidis*

Drug (Generic Name)	Age of contact	Dose/regimen/duration
Rifampin (orally)	Adult	600 mg every 12 h for 2 days
	1 month to 12 years	10 mg/kg every 12 h for 2 days
	Less than 1 month	5 mg/kg every 12 h for 2 days
Ciprofloxacin	Adult	500 mg single dose
Sulfadiazine (orally) (only if index case is known to be susceptible)	Adult	1 g every 12 h for 2 days
	1–12 years	500 mg every 12 h for 2 days
	Less than 1 year	500 mg every 24 h for 2 days
Ceftriaxone (intramuscularly)[a]	15 years or older	250 mg as a single injection
	Less than 15 years	125 mg as a single injection

[a]Ceftriaxone has shown to be very effective for elimination of meningococcal carriage in contacts of cases with group A meningococcal strains but there is a lack of data on reduction of the rate of secondary cases.[144,145]

of age. In the United States this vaccine is currently recommended for routine immunization of adolescents (see Sections 9.3 and 9.3.1).

3. Methodology

3.1. Sources of Mortality Data

The principal sources of mortality data on meningococcal diseases are local and federal health agencies such as, in the United States, local and state health departments and the Centers for Disease Control and Prevention (CDC) through the *Morbidity and Mortality Weekly Report* (MMWR). In addition, there are publications of the World Health Organization (WHO) and various military sources, but these deal only with the populations that they serve. By and large, mortality data reflect the incidence of the disease, although they can be affected by a number of variables, especially the level of health care available, age, and the rapidity with which treatment can be instituted. The level of completeness of mortality data is reflected to a great extent by how well public health reporting is organized and whether adequate laboratory facilities are available for identifying individual disease-causing agents.

3.2. Sources of Morbidity Data

The disease is generally reportable, and because of the fear that it engenders, reporting is reasonably complete and tends to mirror the availability of proper laboratory facilities to identify etiologic agents in individual cases.

Prevalence and incidence data are regularly supplied in the CDC MMWRs and in the *Annual Summary* of the same publication (Figure 1).[27] Additional sources of information are the *WHO Weekly Epidemiological Record* and the *Canada Diseases Weekly Report*. Other data may be found in the published reports describing individual outbreaks. The latter generally observe geographic limitations, whereas the MMWR considers the United States as a whole. Additional data may be found in the reports of various state and local health departments.

3.3. Surveys

Carrier surveys were often conducted among military populations, whereas among civilians, case contacts are often cultured, especially in schools and families. Carrier surveys are primarily of the posterior nasopharyngeal carrier state. In recent years, carrier surveys have been deemphasized.

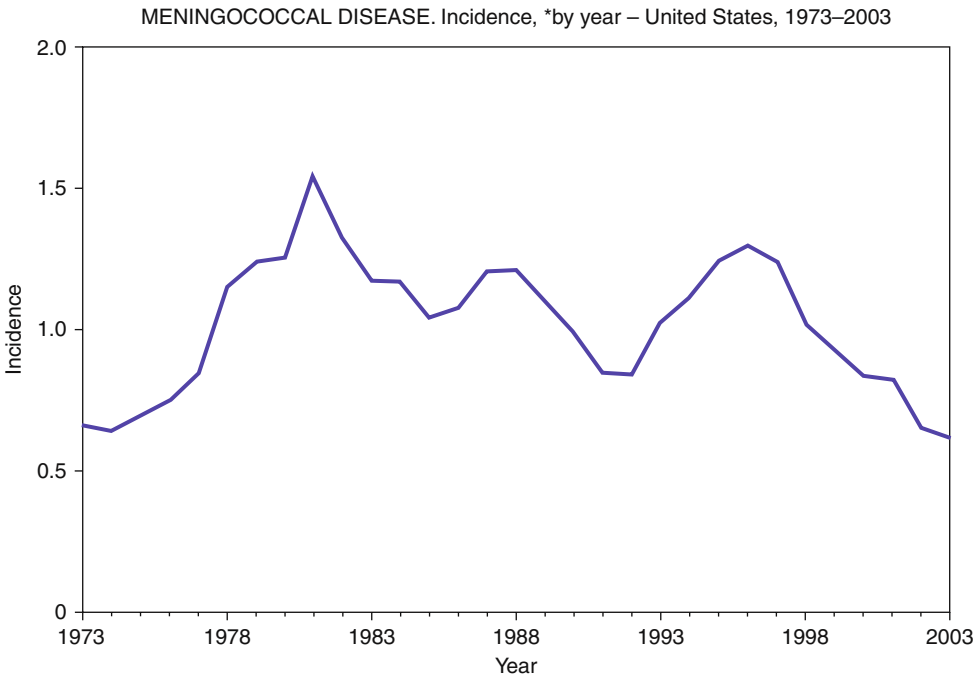


Figure 1. Meningococcal Disease, by year United States, 1973–2003. From reference (27).

This is because they generally yield little information about the immune state of the population or the introduction of new, virulent meningococcal strains. These appear to be more predictive of risk for an outbreak. Serological surveys, other than in vaccination trials, have not been used generally because the available methods do not lend themselves to mass application and, furthermore, because one would have to test for antibodies to all known serogroups.

In the United States, the CDC has sponsored special surveys of meningococcemia and meningitis. These usually target representative states and then are extrapolated to calculate rates for the whole country.[28–30] In 2005 the British Health Protection Agency has published meningococcal disease rates to demonstrate the reduction of serogroup C disease following widespread use of the conjugate meningococcal C vaccine.[31]

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organism. *N. meningitidis* is a fastidious, gram-negative diplococcus that grows best on enriched media such as blood or chocolate agar and in the presence of increased CO_2. The latter is especially useful for primary isolation. The organism may be isolated from all body areas and orifices. When *N. meningitidis* is sought in places where competing bacteria are prominent, it is best to use Thayer–Martin medium,[32] which consists of chocolate agar to which several antibiotics inhibitory for gram-negative, gram-positive, and yeast-contaminating organisms have been added. This medium is especially useful for the identification of meningococci in nasopharyngeal secretions and cultures from rectal swabs. Organisms may be cultivated frequently from the male urethra, the cervix, the conjunctivae, and in cases of arthritis, from purulent septic joint fluids. In patients with meningococcal bacteremia or meningitis, cultures of the blood are usually positive, as are those of spinal fluid. Meningococci may be demonstrated in Gram-stained smears from petechiae or the buffy coats of those with severe sepsis. Cultures from normally sterile fluids such as blood, cerebrospinal fluid (CSF), or joint fluid may be cultivated on regular blood or chocolate agar rather than Thayer–Martin medium, as inhibition of normal flora is not necessary. The same is true of subcultures of individual colonies from primary isolation media.

The organism ferments glucose and maltose but not lactose. Sometimes the fermentation of glucose is slow and may require 48 h or more of incubation. More rarely, glucose may not be fermented at all. Meningococci are oxidase positive, and this reaction may be helpful in the recognition of colonies on plates with mixed flora. The use of Thayer–Martin medium usually removes the latter requirement. The organism does not grow at room temperature or on simple media such as nutrient agar.

Meningococcal polysaccharide antigens can be detected in body fluids or in the supernatant of growing cultures. Latex beads coated with meningococcal antisera are available commercially. They will agglutinate in the presence of soluble meningococcal antigens.

In recent years polymerase chain reaction has been reported to be useful for the diagnosis and serologic determination of *N. meningitidis* from clinical samples.[33–36] Advantages of this technique are theoretical increase in sensitivity over culture and ability to identify the organism in samples in which the organism has been killed because the patient was receiving antibiotics before the sample was taken. The specificity of PCR for *N. meningitidis* is still being studied. Differences in results from various studies may have to do with different genes included in the primers.

3.4.2. Serological and Immunologic Diagnostic Methods. While meningococci contain several antigens, the group-specific one is a polysaccharide that is readily detected by slide agglutination. This usually works well with isolates from body fluids of actual cases. Often, throat and sputum isolates are rough and tend to agglutinate spontaneously in saline. They are also agglutinated in normal rabbit serum and all, or nearly all, group-specific antisera. Such organisms are usually identified as "saline agglutinable." Slide agglutination can be performed rapidly, but requires pure cultures and active antisera. These can be done in fairly large numbers on large plates, and thus lend themselves well to population studies and epidemiological investigations. Organisms of serogroups A and C are encapsulated; when freshly isolated or still in original spinal fluids they may be shown to "quell" with potent antisera. This reaction can be used for rapid identification, especially of organisms in spinal fluid. For serogrouping in a reference laboratory, the hemagglutination inhibition test has been reported to be both highly sensitive and specific.[37] As mentioned in Section 3.4, polymerase chain reaction can be used not only for detection *of N. meningitidis* but also for serogrouping, using serogroup-specific primers such as capsule biosynthesis genes.[33–36]

4. Biological Characteristics of the Organism

Under the special conditions that lead to epidemics, encapsulated (virulent) organisms are transmitted rapidly from person-to-person, probably in respiratory droplets. They tend to localize primarily in the posterior nasopharynx.

Thus, attempts to determine the extent, or degree, of infection of a population at any given time require surveys of the posterior nasopharyngeal prevalence of the organism. This is best achieved with a bent wire swab that is passed up behind the uvula, wiped across the posterior nasopharynx, withdrawn, and immediately plated on Thayer–Martin chocolate agar. The warmed plates should be placed in an incubator as soon as possible thereafter. If the incubator contains CO_2, no other container needs to be used. If no CO_2 incubator is available, then the newly inoculated plates should be put into candle jars. The spontaneous extinction of the candle flame will indicate that the CO_2 content is elevated; when the jar is put at 37°C, suitable growth for further subinoculation and isolation should appear overnight.

Most of the major epidemics of this century have been caused by group A meningococci, although the massive Brazilian epidemic of the 1970s began as a group C outbreak that was then displaced by group A organisms. Localized outbreaks due to meningococci of groups B, C, W-135, or Y have been noted, but more often than not, they are present in sporadic cases.

4.1. Serotypes and Serogroups

Meningococci are grouped according to their polysaccharide capsule antigens into serogroups A, B, C, D, H, I, K, L, X, Y, Z, W135, and 29E. In the United States serogroup A is very rare. Serogroups B, C and Y have been responsible for approximately one-third of cases each, in recent years. In young children under 1 year of age serogroup B is more common, accounting for over 50% of meningococcal disease.[38] The next most common serogroups were W135 and Y, accounting for 3 and 2% of isolates, respectively.[38,39] Fewer than 1% are serogroup A. In contrast, in sub-Saharan Africa and Scandinavia serogroups A and C are predominate. Serogroup W-135, previously found only in carriers, was found to cause occasional cases of meningococcal disease in the 1970s and in recent decades has been associated with outbreaks in Asia, especially associated with the gathering at the Hajj.[14–17]

Meningococci can also be typed according to protein and lipo-oligosaccharide (LOS) antigens. The outer membrane of *N. meningitidis* contains LOS, similar to the lipopolysaccharides of gram-negative bacilli. The outer membrane proteins (serotypes) are shared by isolates from different serogroups. These protein antigens are recognized by the human immune system and they may be developed into effective vaccines.

A number of systems have been devised for typing meningococci.[40,41] Three methods were reported in the 1970s. Zollinger and Mandrell[42] used both outer membrane protein and LOS to serotype meningococci, but with specific absorptions they could type according to outer membrane proteins. Subsequently, Apicella[43] also used LOS to serotype group B meningococci. Frasch and associates[44] subsequently proposed a scheme based on electrophoretic mobility in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels of two major proteins and LOS into 21 serotypes, bringing together schema of several laboratories into a unified scheme. Most of these 21 types can be distinguished by monoclonal antibodies.[44] Multilocus enzyme electrophoresis is a more recently described method used by a number of laboratories. Achtman[45] has utilized mobility of bacterial isoenzyme proteins in an electrophoretic field and typed epidemic strains and has traced clones of serogroup A that have spread from country to country. Ashton et al.[46] used typing by monoclonal antibodies to differentiate serogroup B isolates from Canada using an enzyme-linked immunosorbent assay (ELISA) technique. These techniques are not generally available for typing strains from individual cases, but when applied by research laboratories can provide additional information for epidemiological studies of transmission, relationships among cases, or the presence of an epidemic strain in a geographically defined area.[41,45–48]

4.2. Antibiotic Susceptibility

The susceptibility and resistance of meningococci to various chemotherapeutic and antibiotic agents have played a significant role in the treatment of individual patients and, perhaps more important, in the management of epidemics. The new era began in 1941 with a publication by Dingle et al.[49] that demonstrated that sulfadiazine was effective in the treatment of meningococcal meningitis. This finding was subsequently supported by others.[50] It was also found during these clinical studies that meningococci were eradicated from the posterior nasopharynx within hours after treatment was initiated. Then came a number of trials in the military in which it was observed that small doses of sulfadiazine given for short periods of time were sufficient to eradicate the carrier state.[18–20,51] When 1 g was given twice daily for 2 days to military recruits en masse in a given installation, not only was the carrier rate reduced to negligible levels but also the effect on disease was almost instantaneous. This simple control measure was instituted as a routine in all military installations as well as in civilian populations with excessive numbers of cases or to prevent secondary cases within households.

All this came to an end in 1963 when such control measures seemed to have no impact on the occurrence of

cases at the San Diego Naval Training Center.[22] Investigation of this event led to the demonstration by Millar et al.[22] that resistance to sulfonamides was widespread throughout the United States in both military and civilian populations. Initially, this seemed to be limited to serogroup B and C meningococci, whereas group A infections that were occurring in Africa and in some parts of the Mediterranean Basin continued to be susceptible. However, in a widespread group A epidemic in Morocco,[5] the causative organisms were found to be significantly resistant to sulfadiazine. This was soon confirmed in other parts of the world and led to the recommendation that cases be treated with large doses of penicillin. This has proven to be successful. Subsequently, rifampin or minocycline or a combination thereof has been demonstrated to reduce carrier rates.[52–55]

For decades, penicillin has been considered to be the antibiotic of choice for treatment of meningococcal disease, although other semisynthetic penicillins and cephalosporins and chloramphenicol are also effective. Beginning in the 1980s there have been reports of meningococcal isolates with reduced susceptibility to penicillin. While susceptible strains have a minimum inhibitory concentration (MIC) of 0.03 μg/ml to penicillin, relative resistant strains with an MIC of 0.1–1.28 μg/ml have been reported from Spain,[56] England,[57] South Africa,[58] Canada,[59] and the United States.[60,61] While an estimated 4% of meningococcal isolates in the United States in 1991 were relatively resistant, the clinical significance of resistance at this level is uncertain if large doses of penicillin are used for treatment.[61] Penicillin-resistant strains are generally susceptible to third-generation cephalosporins and these are frequently employed at the beginning of antibiotic therapy before the organism has been positively identified. Penicillin resistance is not due to production of penicillinase by the organism but by reduced affinity of cell wall penicillin-binding proteins (particularly penicillin-binding protein 2) for penicillin.[62] In Spain, where the prevalence of such isolates climbed to 20% in the late 1980s, the types and subtypes of these isolates were quite diverse.[48] Concerns about changes in susceptibility of meningococci to antibiotics has continued in the 2000s.[63] Since the reports showing that ciprofloxacin can be used for meningococcal prophylaxis, resistant to this agent has been reported.[64] In a recent report in which 442 international meningococcal strains were tested, elevated penicillin MIC ($\geq$12 μg/ml and $\geq$25 μg/ml) occurred in 14.3 and 8.6% of strains, respectively, and in addition 13 strains showed reduced susceptibility to tetracyclines, 2 strains had reduced susceptibility to quinolones, and 21.7% were resistant to sulfisoxazole.[65]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Information on prevalence and incidence is complicated by the multiplicity of serogroups of meningococci. Prevalence and incidence are more precisely stated and of greater value when serogroups are identified than when they are categorized under the generic term "meningococci."

In general, the rate of meningococcal disease in the United States has been 0.5–1.1/100,000.[38] Jacobson et al.[66] reported that in the United States in 1974, there was 0.60 case/100,000 population per year. As noted in Figure 1, this was a year of low incidence. Yet, despite this low incidence, the rates were 9.47 in infants less than 1 year old and 2.27 in children 1–4 years old.

In an active surveillance program sponsored by the CDC in 1978, the annual incidence of meningococcal meningitis in the United States was estimated to be 0.72 case/100,000 population. The peak age of meningococcal meningitis was 3–8 months (peak incidence 13/100,000). The peak age of meningococcemia was 6–8 months (peak incidence 5/100,000).[28] A CDC-sponsored laboratory surveillance reported an average annual incidence of 1.1/100,000 in 1989–1991.[39] The highest rate was in infants $<$4 months of age, 26.4/100,000. In 1990–1994, the rate of meningococcal meningitis averaged 0.96 cases per 100,000.[67] In the most recent report, from 1991 to 2002 the rate of meningococcal disease in infants under 1 year old was 9.2/100,000 and 62% of meningococcal disease occurred among children 11 years old or older.[38] While the rate of meningococcal disease in the United States and other Western countries is 1–3/100,000, in developing countries it is much higher, up to 10–25/100,000.[68]

5.2. Epidemic Behavior and Contagiousness

Glover,[69] in a classic report, concluded that carrier rates were most affected by "overcrowding" of sleeping arrangements. While "standard" accommodations in the British Army had been defined by a Royal Commission in 1861 as 60 ft^2 of floor space (600 ft^3 of air space and 3 ft spacing between beds) per man, this was reduced to 40 ft^2 of floor space and 400 ft^3 of air space soon after the outbreak of World War I because of mobilization pressures. At the end of the third year of the war, he found the space between beds in a distinguished unit to be less than 6 inches. Glover concluded that distances between beds were so important that he could predict the meningococcal carrier rate in a barracks by computing the distance between its beds. If the distance was 1 ft 4 in., the carrier rate would be 10%. A slight decrease

sent the carrier rate up to between 10 and 20%. If the beds were closer than 1 ft, 20% was the rate, and if the distance was less than 9 inches, a carrier rate of 28–30% was to be expected. When the recommended distance was maintained, the carrier rate rarely exceeded 5%.

"Overcrowding" (defined as less than 40 ft^2 per man) was soon followed by a sharp rise in the carrier rate, reaching its maximum in about 2 weeks. The rise was accompanied by an increase in the proportion of epidemic carrier strains. The increase of this rate among noncontact carriers to 20% meant that an outbreak was imminent. The high carrier rates could be reduced, although not as quickly as they had increased, by increasing the space between beds to $2\frac{1}{2}$ ft or more. Thus, he concluded that "to raise the carrier rate by overcrowding was both easier and quicker than to diminish it by spacing out."

In the devastating epidemic of 5,885 cases that occurred in Santiago, Chile, during 1941–1942, Pizzi[70] reported that in the area of the city where the epidemic was concentrated, the population density was seven persons per room and 2.9 per bed. The secondary attack rate overall was 2.5%, but it was 3.9% for those under 15 years of age and 1.5% among those more than 15 years of age. This experience tended to support Glover's observations.

Various studies have been reported by other authors in which no cases were seen when carrier rates were high[71] or cases were seen when carrier rates were low. Perhaps a key observation was supplied by Schoenbach,[72] who cultured 200 men in a military unit three times a week for 10 weeks. While the carrier rate averaged 41% during this period, 91% were identified at some time to be carriers. Similar, but unpublished, observations were made by Dr. Harry A. Feldman in conjunction with colleagues at the Fourth Service Command Medical Laboratory at Fort McPherson, Georgia, during the same period.

A source of great puzzlement for public health workers, epidemiologists, and microbiologists who have studied epidemics of meningococcal disease over the years has been the apparent discontinuous pattern of disease occurrence. Arkwright,[73] in a truly remarkable albeit much neglected report, concluded that

> The large number and widespread distribution of carriers and the small number of cases of meningitis compared to the population forces upon one the view that for a true understanding of an epidemic of meningitis, the total number of persons who harbour the meningococcus should be looked upon as constituting the true epidemic . . . the carriers constitute the mass of the epidemic, and those who subsequently develop meningitis are only a small accidental minority. The cases of meningitis, on account of the severity and fatality of this complication, give to the epidemic its importance, and they necessarily bulk largely in the eyes of the clinician . . . a consideration of all the evidence about carriers leads to the conclusion that the statement that cases of meningitis occur when and where carriers of the meningococcus become numerous, is a more correct way of stating the facts than by saying that many carriers occur in the neighbourhood of cases of meningitis.

The meningococcal situation parallels that of poliomyelitis, in which paralytic cases seemed to occur without rhyme or reason until the presence and importance of the nonparalytic individual was established. It was then determined that only about 1 in 100 infected individuals developed paralytic disease.[74] Maxcy[75] concluded that a similar rate occurred in meningococcal infections.

The key to the occurrence of an epidemic is the carrier or acquisition incidence rate. This has to be rapid for an epidemic to happen, since many individuals must acquire the organism for the relatively few cases to occur. It is the concentration of the latter that defines the presence of an epidemic. The key question remains, namely, what initiates the transmission speedup in a population that results in increased numbers of cases and an epidemic? While the carrier state may persist for months,[76,77] the period of communicability is unknown.

It would seem logical to infer that if there were *no* carriers, there would be no cases. This concept led many to investigate the effectiveness of sulfonamides for the elimination of the carrier state, since it had been observed early in the research on sulfadiazine treatment that organisms disappeared rapidly from the posterior nasopharynx.[18–20,51,72] Typical of reports confirming this effective measure was that of Kuhns et al.[20] A simple 2-g dose reduced the carrier rate to near zero, but the effect was prolonged when such treatment was maintained for 2–3 days.[72] Cases disappeared just as promptly.

During World War II, an epidemic in the military was defined as the occurrence of 1 or more cases/10,000 soldiers per week.[78] The epidemic that accompanied World War II can be readily seen in Figure 2, as can the relative increases noted during the Korean and Vietnam conflicts. The latter two reflect recruit outbreaks primarily, while the World War II increase was noted both in the military and in the civilians, worldwide.

5.3. Geographic Distribution

Meningococcal infections are limited to humans and have been noted throughout the world in either small circumscribed concentrations[79] of cases or widespread areas[80–84] involving whole countries (Brazil) or geographically large areas such as sub-Sahara Africa.[4] In 1948–1950, there was an epidemic of 92,964 cases with 14,273 deaths in northern Nigeria.[85] The band of countries stretching across sub-Sahara Africa from

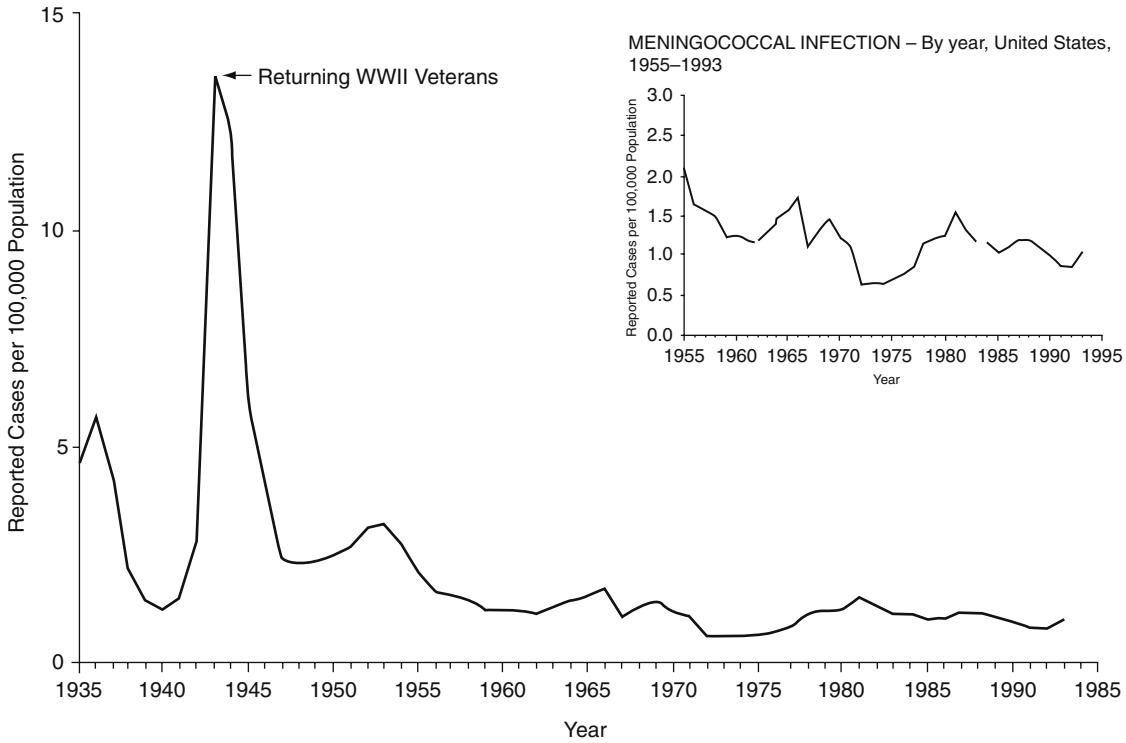


Figure 2. Meningococcal Infection — by year, United States, 1935–1993.
Source: Centers for Disease Control and Prevention. Summary of notifiable diseases, United States, 1993. MMWR 1993;42, 41

Gambia to Ethiopia is often referred to as the meningitis belt and epidemics in this area are frequent, with rates of meningococcal disease of up to 1,000/100,000 (1%).[68] In recent years, outbreaks of meningococcal disease have occurred in Saudi Arabia at the time of the *Hajj*, presumably due both to influx of individuals who carried epidemic strains and to overcrowding. A major outbreak occurred in 1987 with a particular clone of group A that was spread by pilgrims returning from Mecca to many other countries.[45] In the early 2000s meningococcal serogroup W-135 has been responsible for the *Hajj*-associated outbreaks. Many health authorities have recommended that travelers to Mecca receive meningococcal vaccine.[86] Travelers now require meningococcal vaccine and this appears to be responsible for a current reduction in the *Hajj*-associated meningococcal risk.

5.4. Temporal Distribution

Sporadic endemic cases of meningococcal infections are encountered throughout the world at all times. Outbreaks tend to occur in various places for no discernible reason(s). On occasion, epidemics break out almost simultaneously in different places with no apparent connection with each other.

In tropical areas, especially sub-Sahara Africa, cases are most often seen during dry seasons, perhaps because changes in sleeping arrangements accompany the regression of the rainy season. In the dry season the dusty conditions drive workers indoors to sleep in crowded rooms.[80] In the cooler temperate zones, outbreaks tend to be clustered in the winter and spring months.[87] Recent data from Burkina Faso and Niger show annual peaks in the summer and fall.[88] In the United States, the peak incidence of meningococcal disease is in the late winter and early spring (February through May), and the lowest incidence is from July through October.

5.5. Age

Basically, meningitis is a childhood disease most often seen under age 15 years.[50,71,89] Beeson and Westerman,[90] in a study of 3,575 British cases, found that 45.5% occurred in children under 15 years. In the great Detroit epidemic,[91] children under 15 years made up 60% of the patients, while in the German epidemic of 1905–1907, 80% were under 15 years.[90] Recent US data confirm that the age-related predisposition of meningococcal meningitis still exists and that the peak incidence is from age 1 month to 1 year (Figure 3)

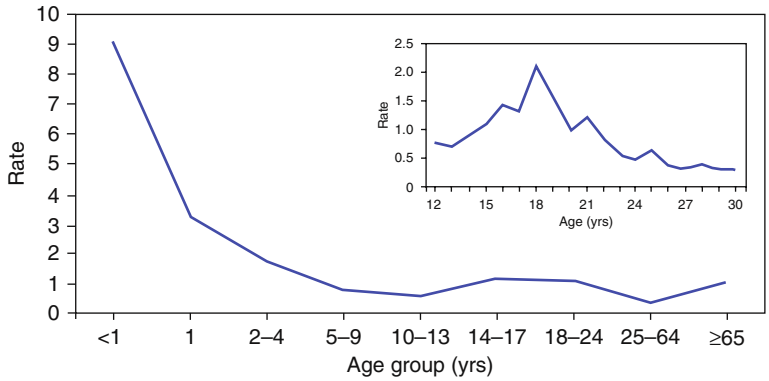


Figure 3. Rate* of meningococcal disease, by age — United States, 1991–2002. From reference (38).
Source: Active Bacterial Core Surveillance data.
*Per 100,000 populations.

but that a lower peak is seen in late adolescence. The early peak in infancy is believed to be due to lack of protective antibody, while the peak for adolescents is believed to be due to exposure to carriers in crowds and in poorly ventilated locations such as bars and dormitories.

5.6. Sex

As is true of other severe diseases, males are more often subject to meningitis than females. In the Beeson and Westerman[90] study, the male–female ratio was 6:4, but fatalities were more frequent in females. The excess of males persisted through age 50 years. This preponderance was also noted in the Detroit civilian outbreak.[91] Since military forces in the past consisted predominantly of males, an excess prevalence of males is to be expected. Thus, in time of war, one should expect male cases to predominate, especially among recruits.

In a longitudinal study of meningococcal carriage in a population of "normal" families, it was found that males 20 years or older were more likely than adult females to become carriers.[76] However, recent data (2003) from the CDC show that 50% of cases of meningococcal disease occurred in males (annual rate in males = 0.60/100,000 and in females = 0.61/100,000[27]).

5.7. Race

Race seems to be of little significance for either the acquisition or the severity of infections, except that those with sickle-cell disease may be more prone to fatal infections, as they are to those caused by other polysaccharide-encapsulated bacteria. In a review of deaths from bacterial meningitis in the United States from 1962 to 1968,[92] it was concluded that excessive deaths from this cause were noted among African Americans and American Indians. Race per se is not mentioned as a risk factor, but poverty and crowding are. In a population-based study in Charleston County, South Carolina, neither race nor income affected the risk for meningococcal meningitis.[93] In the multistate surveillance study performed by the CDC in 1986, the rate of meningitis in African Americans caused by *N. meningitidis* was twice that of whites (1.5 vs 0.7/100,000), with Hispanics having a rate in between (1.1/100,000).[29] National data from the CDC for 2003 continue to show a higher annual incidence of meningococcal disease in African Americans (0.63/100,000) compared with whites (0.46/100,000). The rates for Asian Pacific Islanders (0.27/100,000) and for American Indians/Native Alaskans (0.26/100,000) are based on very small numbers. In terms of ethnicity, the rate for Hispanics (0.50/100,000) is higher than that for non-Hispanics (0.38/100,000).[27]

5.8. Occupation

Pike,[94] in an exhaustive review of laboratory-associated infections, records two such fatalities. In 1918, an employee of the State Serum Institute, Copenhagen, Denmark, whose job was concerned with the production of antimeningococcal serum, died of meningococcal meningitis. Another, an employee of the National Institutes of Health, in the United States, while injecting an animal with meningococci, had some of the syringe's contents sprayed into her eyes as the animal struggled. She became ill 4 days later and died after 4 days of illness.

The CDC reported two cases of fatal disease in 1988 in bacteriologists from California and Massachusetts who had handled meningococci in a laboratory, each having the

same isoenzyme type as a strain isolated from them at the time of their infection. There was considerable evidence of laboratory-acquired infection.[95]

While these cases indicate a potential occupational hazard, symptomatic infections with this organism are exceedingly rare. Nonetheless, it is prudent that laboratory personnel receive the tetravalent meningococcal polysaccharide or conjugate vaccine before taking up their work assignments and that laboratory workers who work with large quantities of organisms be protected with gloves and laboratory coats and use a class II biologic safety cabinet in a biosafety level 3 laboratory. Persons exposed to high-concentration aerosols or who have percutaneous exposures should have prophylaxis with treatment doses of penicillin.[7]

There are no other identifiable occupational hazards except for military recruits during periods of mobilization, who seem to have a special susceptibility that becomes manifest in outbreaks among them. A relatively high rate of meningococcal disease in college freshman living in dormitories suggests that age, crowding, and lifestyle may account for a peak of infection in military-aged individuals.

Despite great fears and expressed anxieties, physicians and nurses tend not to be excessively susceptible either to sporadic cases or during outbreaks. Arkwright[73] stated:

> It is almost universally accepted that doctors and hospital nurses seldom contract the disease. These and similar facts which have been observed in many epidemics are evidence against the spread of the disease being usually due to direct infection from one patient to another.

This concept has been modified in the present era by two new therapeutic procedures. Several cases of meningococcal disease have occurred among physicians who gave mouth-to-mouth resuscitation to patients severely affected by such infections. Feldman reported a case in which infection was apparently transferred with a kidney transplanted from an undiagnosed fatal case to a susceptible recipient.[96] Other than in the case of direct exposure to respiratory tract secretions of infected patients such as with mouth-to-mouth resuscitation, medical personnel do not require prophylaxis following the delivery of medical care to patients with meningococcal disease.

5.9. Occurrence in Different Settings

The most predictable outbreaks have been those that accompanied increased military mobilization and the induction of large numbers of recruits such as were noted in World Wars I and II and the Korean and Vietnamese mobilizations in the United States. The most extensive and largest outbreaks in the 20th century were those in civilians in sub-Saharan Africa and in Brazil. While more than a quarter of a million cases occurred in the latter, even under such conditions, outbreaks are spotty and concentrated in the poor and crowded;[54,70] e.g., during the Vietnamese mobilization, extensive outbreaks occurred among US Army and Navy recruits, while the Air Force and Marines experienced only sporadic cases and were spared the major epidemics that affected the other two services. One would assume that the induction system being what it is, susceptibles would have been distributed more or less equally among all four services. Cases were almost unknown among female recruits, but there were many fewer of them and their housing conditions were relatively uncrowded. Outbreaks of meningococcal disease in the military has not been a problem since the development of the polysaccharide meningococcal vaccine and its widespread use in the military throughout the world in the early 1970s.

Daniels et al.[97] reported that whereas a third of the troops at Fort Bragg, North Carolina, had less than 3 months of service, 59% of all patients with meningococcal infections were in this category. In other places, as many as 90% of cases were reported among recruits with less than 90 days of service. In the military, then, meningococcal infections represent a disease of recruits, whereas among seasoned troops or cadres in training, only sporadic cases are likely to be seen.[78] These are probably the result of deficiency of antibodies to specific circulating meningococcal strains.[98] A similar situation has been noted among South African miners, who represent a young population in whom meningococcal infections tend to occur early in their employment in the mines. Those who reenlist for a second tour of service are seldom troubled by this disease. Outbreaks may also be noted on occasion in institutions such as jails, mental asylums, residential schools, and other closed populations.

Outbreaks of meningococcal infection are quite uncommon in day-care centers or other infant-care facilities, but they have been reported, usually as part of a community outbreak. A report from the CDC, published in 1977,[99] described a cluster of three cases of serogroup B disease from a private home day-care center with nine attendees in Georgia. Review of the recent literature at that time revealed a cluster of three group B cases in a crowded church nursery attended by 40 children under 6 years of age in 1966–1967, and two group C cases in a day-care center in Dade County, Florida during an epidemic in 1969–1970.[99]

In 1981, there was a large outbreak of meningococcal disease in an elementary school classroom in Houston. In this outbreak there were five children and two siblings of one

child who became ill within a 6-day period with serogroup C *N. meningitidis*. This outbreak was unusual in that the children were 12 years old, older than in other reports of spread among children, and that disease was not noted in other classrooms or the community at large. Other studies of cases in school classrooms have been notable for the absence of secondary cases. Overcrowding may have been the major promoting spread of disease.[100]

In 1978 there was an outbreak of *N. meningitidis* serogroup A associated with the gathering at Medina and Mecca, Saudi Arabia, associated with the *Hajj* and more recently, in the years 2000–2001 there have been serious outbreaks of meningococcal disease (primarily serogroup W-135 but also serogroup A.[15–17,45]

5.10. Socioeconomic Factors

Outbreaks among civilians tend to occur among the poor, especially those who are overcrowded, ill-housed, and ill-fed.[70] In the great Brazilian epidemic of the 1970s, cases were concentrated in the barrios and typically were unusual among the middle and upper classes. Thus, even within cities, cases are often localized and confined to small areas rather than spread among the community as a whole. In a surveillance study of bacterial meningitis in Charleston County, South Carolina from 1961 to 1971, neither race nor income affected the risk of meningococcal meningitis, even though income was inversely correlated with rate of meningitis due to *Haemophilus influenzae* and *Streptococcus pneumoniae*.[93]

5.11. Other Factors

The principal factors that seem to be related to the occurrence of meningococcal infections are climatic conditions. In the sub-Sahara area, the change from the wet to the dry season tends to lead to an increase in cases. The reason for this relationship is unknown, although Belcher et al.[80] are of the opinion that considerably more population mixing takes place during the dry season, when farming work is reduced and more time is available for socializing and visiting. Also, there is a shift from sleeping outdoors to sleeping indoors in crowded and poorly ventilated rooms. In northern Ghana, the peak occurrence of cases comes during the dry season, when there is little rainfall, low humidity, and cold nights. Since this is the time when food becomes scarcer, it is popularly known as the "hunger season."

Familial and genetic factors may play a significant role in the acquisition of meningococcal disease. One example is that among those who lack one of the terminal complement components there is increased risk of infections due to neisserial species[101] (see Section 8.1.1).

6. Mechanisms and Routes of Transmission

Transmission of meningococcal infections is usually assumed to be by way of droplets from the respiratory tract, regardless of the clinical status of the individual. Organisms are present in almost pure culture in the posterior nasopharynx of colonized individuals. Direct transmission may occur during mouth-to-mouth resuscitation, by the transfer of infected blood, such as an accidental needle puncture from a contaminated syringe, or by organ transplant. Nosocomial transmission, while probably very rare, was documented by Cohen et al.[102] in New Haven, Connecticut. At a time when two patients in a cancer ward developed group Y meningococcal infections, the prevalence of asymptomatic pharyngeal carriage of group Y strains was 10% among other patients on the ward. The carriage prevalence was significantly lower in ward workers and casual visitors. Spread was presumably due to airborne transmission.

Acute, purulent meningococcal urethritis[103] has been reported along with reports of isolations of meningococci from the cervix[104,105] and other unusual areas.[106–114] How often these reflect sexual activity or generalized infection is unclear. It has been suggested that cervical acquisitions may be related to orogenital sex, or perhaps transmitted by a male with meningococcal urethritis, or the result of generalized dissemination during mild meningococcemia. Whether direct cervical implantation leads to clinical systemic disease is unknown.

6.1. Secondary Attack Rate

Considerable confusion has surrounded the calculation and interpretation of secondary attack rates of meningococcal disease. Norton,[115] in his study of the meningococcal epidemic of 1929 in Detroit, found among the 724 cases of meningitis, 32 (4.4%) additional cases in households. Among these additional cases, he believed that 2 could be eliminated completely, leading to the conclusion that there had been 30 secondary household cases. In the Santiago, Chile epidemic, Pizzi[70] calculated the secondary attack rate to be 3.9% for those less than 15 years of age and 1.5% for those older than 15. For all ages during this epidemic of 5,885 cases, the secondary attack rate was 2.5%. During the massive Brazilian epidemic, a study[116] conducted among classmates of children who had meningococcal disease was

interpreted as indicating that classroom exposure to cases did not increase the risk of acquiring meningococcal meningitis. The authors concluded that such exposure was an insufficient indication for prophylactic chemotherapy.

The Meningococcal Disease Surveillance Group,[117] organized by the CDC, analyzed 326 cases reported in the United States from November 1973 through March 1974. Three household members manifested meningococcal disease following initial cases in their homes, a secondary attack rate of approximately 3/1,000 household members at risk. Among 293 households for whom follow-up data were available, 4 had two cases and none had more than two. In this study, cases in contacts with onset within 24 h of the case to which they were exposed were labeled "co-primary." In 107 households in which no chemoprophylaxis was administered to any household members, the secondary attack rate was 2.2/1,000. The authors concluded that a secondary attack rate of 2–4/1,000 is still a thousand times greater than the overall reported rate of 0.23/100,000 of meningococcal disease in the states in which the cases were studied.

Clearly data on secondary attack rates during nonepidemic periods estimate lower rates than in earlier studies which were performed during outbreaks. A recent review by Purcell et al.[118] looked at studies performed since the early 1970s that calculated the attack rate for household contacts who did or did not receive antibiotic prophylaxis. The attack rates varied from 0 to 5/1,000.

It is not always clear whether a distinction is drawn between secondary and co-primary cases, as was attempted by Leedom et al.[119] in a 3-year study of Los Angeles County cases. There were 290 different families, 3.4% of which had more than one case. From a sample of these cases, it was determined that the average number of people residing in the same household with each patient was 3.71. The risk of multiple cases among household contacts of patients was estimated to be 992/100,000. The 3-year case average (1963–1965) for Los Angeles County was 2.09/100,000. One household had three cases. The onset of disease was basically simultaneous among all family members except for three in which the cases were separated by 5, 7, and 9 days. The remainder, then, would be considered to have been co-primary cases. The high rate of secondary cases during epidemics compared with the rate with endemic disease may be due to multiple factors, but the problem of assigning status as a true secondary case may be more difficult during an epidemic.

What, then, are the implications of using these different designations, or is it solely a matter of semantics? The incubation period for meningococcal disease varies from 2 to 10 days, commonly 3–4 days.[120] Bolduan and Goodwin,[121] in a 1905 study in New York City, identified 88 instances where there was more than one case in a house. Among 58 of these, the interval between the index and the second case was 7 days in 14 and 2 weeks to 3 months in 16. Norton[115] stated that among 6,416 household contacts of 1,272 cases, there were 36 homes with more than one case. In three, two persons became ill the same day, and in another, there were four simultaneous onsets. The latter had a fifth case sometime later. Norton calculated that while 3.6% of homes had additional cases after their first, there were 46 (0.7%) secondary cases among 6,416 contacts. Of these 46, 20 had their onsets within 4 days and 14 within the next 5 days, or 34 (74%) within 9 days of the index patient. Thus, with an incubation period up to 10 days, most of these "secondary" cases may be really co-primary. In contrast, Jacobson et al.[116] defined a secondary case as "the onset of illness more than 24 h and less than 31 days after onset of another case in the same classroom." Their definition was more applicable to the issue of prophylaxis where it is unlikely that an oral prophylactic agent would affect an already-invasive infection.

7. Pathogenesis and Immunity

Meningococci are generally transmitted via droplets or through close oral contact. The organisms have a predilection for the posterior nasopharynx and can often be obtained in almost pure culture from that area. It was believed for a long time that the bacteria penetrated to the central nervous system through the cribriform plate, but there is no real evidence for this. Dingle and Finland[87] summarized the evidence for and against this concept and decided, as had most observers, that dissemination from the posterior nasopharynx is via the bloodstream. Once the bloodstream has been entered, a variety of consequences may follow, leading to various clinical expressions.[81] As shown in Table 1, these manifestations may run the gamut from mild, self-limited bacteremia—which may or may not be accompanied by low-grade fever, a pinkish, maculopapular rash, arthralgias with malaise, and muscle pains—to overwhelming sepsis with a coalescing, extensive petechial rash that may lead to large areas of necrosis. Disseminated intravascular coagulation, meningitis, septic arthritis, epididymitis, myocarditis, conjunctivitis, adrenal collapse (Waterhouse–Friderichsen syndrome), and death within an hour or two may follow. Fortunately, it is only the rare individual who falls into this latter category. What there is about such individuals that makes them especially susceptible to such severe disease manifestations is unknown, but they arouse the greatest interest and serve to direct attention to the disease. Probably as many as 90% of those who acquire meningococci have infections that are limited to the posterior

nasopharynx with, occasionally perhaps, self-limited bacteremia. Whether infection limited to the posterior nasopharynx leads to clinical signs has been debated for decades and remains unresolved. Unfortunately, most such studies have been performed among military recruits, who are notorious for the amount of bacterial, mycoplasmal, and viral upper-respiratory infections that they support. Each of these agents would have to be excluded before one could conclude that meningococci are producing the reference symptoms. It is the prevalence of posterior nasopharyngeal infections and the paucity of clinical disease that complicate our understanding of the epidemiology of these diseases. That relationship led Arkwright[73] to conclude that epidemics basically affect the nasopharynx and that cases of meningitis and other complications are incidental. This also explains the apparent indiscriminate occurrence of clinical disease and the lack of evidence for direct connections between cases.

Immunity has been studied extensively during the past three decades. Serum antibodies have been demonstrated by a variety of techniques, including radioimmunoassay,[122] bactericidal,[98,123] hemagglutination,[124] agglutination,[123,125] and others. The solid-phase radioimmunoassay is particularly suited to determination of antibody to noncapsular antigens.[126] Immunity is acquired as the result of infection even when this seems to have been limited to the posterior nasopharynx.[123,127] Thus, the carrier state can induce immunity to the serogroup of the organism carried. The presence of bactericidal antibodies correlates well with protection from disease.[98,127] Bactericidal antibody assays are still used for measuring protection in vaccine trials. Because the endemic meningococcal disease rate is so low, vaccine efficacy trials are still reported as inducing a titer of bactericidal antibody and not reduction of disease. The presence of bactericidal antibody to serogroups not in circulation in specific populations suggests the importance of antibody to protein and LOS type-specific antigens that are shared among serogroups.

An alternative hypothesis for the role of antibody, in military recruits, was proposed by Griffiss.[128] He has found antimeningococcal IgA in the acute sera of military recruits who had meningococcal disease. This IgA was able to inhibit the lytic function IgM in sera. Removal of IgA made sera that appeared to lack bactericidal antibody functionally lytic. Thus, it was Griffiss' contention that susceptibility, in addition to being defined by the *absence* of functional antibody, may be due, in some cases, to the *presence* of blocking IgA.[128] Thus, as it is the acquisition of new strains of meningococci that represents the major risk factor for invasive disease, true susceptibility may also be related to exposure to successive waves of meningococci. The role of this mechanism, if any, in civilian endemic disease is unknown.

8. Patterns of Host Response

Inapparent infections play an important role in meningococcal disease because they constitute the majority and account for the bulk of carriers. As has been noted, the acquisition of the carrier state leads to immunity,[98,123] and thus protection against the disease itself. We have no information as to why, among children, an occasional individual is unable to restrict the acquired meningococci to the posterior nasopharynx and becomes the victim of a life-threatening disease episode. Among adults, deficiency of lytic antibody appears to be the major predisposing factor accounting for biological susceptibility to meningococcal disease. The reason why a small number of adults lack antibody is also unknown. Either they were never colonized or colonization did not result in the production of a long-lasting antibody response.

8.1. Clinical Features

Meningococcal infections may manifest themselves clinically by several different syndromes (see Table 1). These may overlap or be noted sequentially in the same patient. The most common occurrence is represented by the asymptomatic acquisition of meningococci in the posterior nasopharynx or the carrier state. While there are some who believe that an acute pharyngitis may be clinically manifest in this stage, this has never really been proven. Thus, disease is probably limited to less than 10% of those who acquire meningococci. The next stage may be that of a mild, self-limited bacteremia that may be accompanied by an evanescent rubella-like rash, arthralgias, fever, malaise, and myalgias. A blood culture taken at this time is often positive for meningococci. This may regress completely or for several days, only to be followed by an explosive onset of severe illness. This is generally heralded or accompanied by a severe petechial rash that may be very extensive and coalescent, with large areas of necrotic skin and muscle. Similar lesions probably occur in the deeper viscera.

Prostration and shock may follow in short order, with death close behind. Smears made from petechial lesions or from the buffy coat of peripheral blood may contain gram-negative diplococci within or outside of cells. This is the most devastating form of the infection, in which septicemia is so severe and overwhelming that often the patient dies before there is localization in the CNS, joints, or other organs.

A less virulent form of septicemia is more common, and while this too is identifiable with a petechial rash, progression is slower and permits other complications to become evident. These patients often are the cases with meningitis, septic arthritis, epididymitis, pneumonitis with pneumonia, conjunctivitis, and myocarditis. All these may occur in a single patient or in any combination thereof.

In infants, "mild" meningococcemia may present with fever as the only symptom. This presentation of meningococcemia is very similar to that of occult pneumonococcemia, which occurs more frequently than meningococcemia in ambulatory pediatric patients.[129,130] So far it has not been possible to differentiate children with meningococcemia who are in the early stages of infection and are destined to develop fulminant disease from those who will clear the infection on their own. A retrospective study to determine whether any hematological parameters differentiated febrile pediatric patients with unsuspected meningococcemia from those with sterile blood cultures only found that the percentage of band forms was higher in those with unsuspected meningococcemia. However, the predictive value of an elevated band count was too low to be of practical value.[131]

Discrete meningococcal pneumonia has also been reported, especially from the military. Group Y meningococcus has often been implicated in this form of infection.[112] Koppes et al.[132] reported on 88 cases of group Y meningococcal disease in Air Force recruits, 68 of whom had primary bacterial pneumonia. All but one did well with penicillin therapy. It is possible that the predominance of group Y in these cases reflected immunization with group C polysaccharide vaccine, which had been instituted as a routine procedure in the Air Force. In this study, 52 of 64 individuals sampled had pure cultures of group Y meningococci in transtracheal aspirates. In the remainder, growth was due primarily to group Y meningococci, with relatively small amounts of contaminants such as α-streptococci, *Haemophilus influenzae* type b, and others. Primary meningococcal pneumonia, either alone or in concert with other evidence of septic meningococcal infections, has been reported often enough to constitute a disease entity, establishment of the identity of which should be sought in cases of otherwise undefined pneumonia.

Group Y meningococcal infections have also occurred in children.[129,133,134] Pneumonia was reported in one case.[134] Group W-135, which has increased in incidence in the past two decades, has also been reported to have a varied presentation in children, similar to other serogroups.[14,135]

There is an unusual and infrequently seen form of the disease that is identified as chronic meningococcemia. This may be a manifestation of subacute bacterial endocarditis or the result of continuous seeding into the bloodstream from an unknown nidus. Such patients may go for weeks or months with recurring fever and evanescent rashes, as either pink macules or small petechiae. Arthritis has frequently been reported with chronic meningococcemia. They respond rapidly to treatment and are often cured by penicillin administered for other reasons, such as a suspected infectious cause of the fever, malaise, and rash.

8.1.1. Genetic Determinants of Meningococcal Disease: Complement Deficiency. Petersen et al.[101] summarized information from various reports of 24 patients who had had recurrent episodes of meningococcal or (generalized) gonorrheal infections. Their distinguishing characteristic is that they lacked one or more of the late components of the complement system: C6, C7, or C8. Of the 24 patients, 6 were deficient in either C6 or C7 and 8 in C8. In all, 13 of the 24 had either *N. meningitidis* or *N. gonorrhoeae* bacteremia, and all recovered. The patient with C7 deficiency apparently recovered from four episodes of meningococcal meningitis. The frequency of this abnormality is unknown, but it would seem that while the propensity to have recurrent neisserial infections is present in such individuals, the disease does not appear to be overwhelming or fatal, since so many seem to have recovered. From subsequent reports it appears that deficiency of C5 and C9 are also risk factors for neisserial infections.

Ellison et al.[136] studied 20 adults with a first episode of meningococcal infection. Six of them had a complement deficiency. Of these six, three had a specific terminal component deficiency and three had multiple component deficiencies due to an underlying illness. In children, the prevalence of underlying complement deficiency in those with one meningococcal infection was 18% in one study.[137] In a larger study, of 544 patients with meningitis or bacteremia due to encapsulated organisms, seven had complement deficiencies; of those seven, six experienced meningococcal infection and one had pneumococcal bacteremia.[138] While the rate of complement deficiencies varies between ethnic groups, the estimate of prevalence is 0.03%. The prevalence of patients with complement deficiencies among those with meningococcal infections is higher when the case is sporadic, in an area of low incidence, than when it is part of a meningococcal outbreak. In a study comparing complement-sufficient patients who had meningococcal disease with patients with complement defects, those with complement defects were older (mean age of 17 years vs. 3 years), more likely to have recurrence and relapse, and had a much lower mortality rate (1.5 vs. 19%).[138] In a recent review Emonts et al.[139] list a number of genetic polymorphisms in the classical complement pathway and in the properdin

innate immunity pathways that increase the risk for invasive meningococcal infections. Immunization with meningococcal polysaccharide vaccine appears to offer some individuals with complement deficiencies protection from meningococcal disease.[140]

Corfield[141] may have recorded the first such case in 1945 and suggests another, seen by Boudin, in whom three episodes occurred in 1849, 1850, and 1851. These cases are to be separated from the recurrent disease that was encountered before modern therapy prevented the frequent bacterial relapses common to that era. Patients who have recurrent disseminated neisserial disease should be screened with CH_{50} assays, and if they are positive, specific deficiencies should be sought.

8.1.2. Other Genetic Determinants of Meningococcal Disease. Recent publications suggest susceptibility to meningococcal disease may be related to mutations and variations in genes controlling toll-like receptors. Smirnova et al.[142] reported that TLR4 variants were over-represented in the population of patients with meningococcal sepsis, while other toll-like receptors appeared to be normal. Other genetic polymorphisms associated with decreased bactericidal capacity with increased susceptibility to meningococcal infections include variants of FCγ receptors, polymorphisms in the tumor necrosis factor$_\alpha$ (TNF$_\alpha$) promoter, and variants in other cytokines including in the interleukin family.[139]

8.2. Diagnosis

As is true of many diseases, the frequency of the diagnosis of meningitis often reflects the observer's index of suspicion. The carrier state is not diagnosable except by culture and is of little importance except under some very special circumstances. In the case of invasive disease, diagnosis generally requires laboratory confirmation. With this in mind, blood cultures should be obtained as quickly as possible. If there are clinical signs of meningitis, an immediate lumbar puncture is mandatory, followed by prompt examination of the fluid. Its cellular content should be ascertained as to type and number. Sugar and protein should be measured (one should determine the comparative blood sugar at the same time.). A Gram's stain should be performed on a smear made from the fluid if the fluid is cloudy; but if it is clear, a concentrated specimen should be made after centrifugation. Fluid should be cultured on chocolate agar in an atmosphere of increased CO_2. While rarely performed today because of the lack of laboratory space on treatment units Gram's stains of smears of fluid from petechial lesions or buffy coats of peripheral blood may also yield gram-negative diplococci. If these are present, then they are most likely meningococci. The peripheral white blood count is usually elevated, but in severely ill patients, it may be less than 4,000/mm^3. If a patient has been treated with antibiotics prior to obtaining samples for culture detection of antigens in blood, urine, or spinal fluid using the latex agglutination test may be helpful in making a diagnosis even if the cultures are sterile. Lack of demand for this test has resulted in its removal from many clinical laboratories. Employment of broad-spectrum antibiotics has reduced the need to make a "bedside" specification.

Because of the nature of the rash and the clinically severe illness, the diagnosis may be confused with Rocky Mountain spotted fever. Less severe cases may be confused with endemic typhus and, when very mild, even with rubella.

9. Control and Prevention

9.1. General Concepts

Because cases of meningococcal disease tend to be distributed indiscriminately in a population and because death may be sudden, the appearance of a case frequently leads to generalized hysteria in the associated population, whether it be open or closed. Outbreaks tend to occur in closed groups such as institutions, recruit military camps, and crowded, impoverished districts of major cities.[6,70] As with poliomyelitis, cases rarely lead to other cases, an observation that understandably tends to increase fear and hysteria among the populace in which the cases occur.

Sporadic cases of meningococcal disease occur throughout the year in all places. They often appear as isolated instances for reasons that are not understood. Close contacts of individuals with meningococcal disease have higher carriage rates than the general population and secondary cases of invasive disease account for 1–3% of all meningococcal cases.[40,143] More incomprehensibly, cases may begin to occur in rapid succession in a given population. These are usually closed, institutionalized settings, but numerous cases may be noted in the community at large and constitute an epidemic.

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis: Suggestions for the Management of Outbreaks

Before rational intervention can be instituted or attempted when it is suspected that cases of meningococcal disease significantly above the endemic rate are occurring, two important questions must be answered. First, can the affected population be defined; and second, what are the characteristics of the causative agent? In respect to the latter, is it of a single serogroup?

Once appropriate antibiotic prophylaxis is given in a large population the carrier rate may be expected to drop to near zero almost immediately, slowly rebuilding itself over a period of weeks. Cases also stop occurring as the carrier rate diminishes. It is required, for maximum benefit, that prophylactic treatment be simultaneous for the total target population. In the past sulfonamides were the first-choice antibiotics for prophylaxis but in much of the world sulfonamide resistance of meningococci has led to the use of alternative antibiotics (Table 2).

Another option is to attempt to immunize the population with a polysaccharide or conjugate meningococcal vaccine. The latter is feasible only if the causative organisms are of serogroups A, C, Y, or W-135. The licensed vaccines contain the polysaccharides of groups A, C, Y, and W-135, and only a single dose is required. When the vaccine is used, it must be anticipated that maximum beneficial effects will be noted after 10–14 days, although diminution in the severity of the disease in some cases may be observed by the 7th day.

Of the other chemoprophylacticagents rifampin is most often used. The dose of rifampin is listed in Table 2. Minocycline, recommended in the past, is rarely used for this indication because of gastrointestinal and vestibular toxicity.[53,54] Resistance to rifampin has been noted in as many as one fourth of strains. This proportion increased with repeated courses of the drug in the same population. A single intramuscular dose of ceftriaxone is effective in eradicating meningococci from the pharynx,[144,145] and it can be used for meningococcal prophylaxis. Its high cost and difficulty of administration limit the usefulness of this option to special circumstances where repeated oral dosing is not practical. Ciprofloxacin is approved for this use as a single dose but resistance has been reported in Europe and Asia but is rare in the U.S. It should not be used in locales where resistance has been reported.

9.3. Immunization

Gotschlich, Goldschneider, Artenstein, and their colleagues,[98,124,127] in the search for an effective alternative to sulfonamide prevention of meningococcal disease, successfully reopened the investigation of vaccines, an approach that had lain dormant for a quarter of a century. A series of publications beginning with the demonstration of the importance of serum antibodies in protection against the disease,[98] the effectiveness of pure carbohydrate antigens[124,127] in stimulating specific immunity against infection with organisms of the various serogroups, and finally the value of such antigenic stimulation in providing protection against the disease during epidemic periods[26] was probably the most important contribution to the meningococcal problem in the twentieth century. Subsequently, group A meningococcal vaccine was used in the management of large epidemics of disease in Finland,[7] Brazil,[6] and Egypt.[146] Effective polysaccharide immunogens for groups Y and W135 appeared later.[147]

Greenwood et al.[148] evaluated the effectiveness of group A vaccine in preventing secondary cases when administered to household contacts of group A cases and found the secondary attack rate to be 18/1,000 among control household contacts who received tetanus toxoid. Among the 520 case contacts who had received group A meningococcal vaccine, there was only one instance of meningococcal disease. The efficacy of the group A meningococcal vaccine was clearly demonstrated in the trial of Peltola et al.[25] in Finland in 1974–1975. In two trials conducted during an epidemic of group A meningococcal disease, 49,295 and 21,007 children, aged 3 months to 5 years, received the vaccine. None developed group A meningococcal disease. In the first trial there were two control groups that had significantly more cases than the vaccine group.

Despite the Finnish experience, there is good evidence that in children the degree of antibody response to the groups A and C vaccine, measured as the concentration of anticapsular antibody in the serum, is proportional to age. Children under 1 year of age have a poor response and no immunologic memory.[149,150] As is seen for other polysaccharide vaccines that are non-T-cell-dependent immunogens, it is not until 2 years of age that there is sufficient maturation of the immune system for there to be a protective antibody response to group C polysaccharide, although the response to the group A polysaccharide appears to occur earlier.[149,150]

Initially, meningococcal vaccine was marketed for civilian use as 50 μg of group A polysaccharide, 50 μg of group C polysaccharide, or a combination of both. In 1981, a tetravalent vaccine consisting of 50 μg each of groups A, C, Y, and W-135 capsular polysaccharides was shown to be safe and immunogenic and has been licensed (Menomune, currently manufactured by Sanofi-Pasteur, Inc.).[151] A single 0.5-ml dose of vaccine is sufficient to immunize a child over 2 years of age or an adult. Adverse reactions have not presented a problem, generally being limited to local erythema or soreness or both, usually persisting for not more than 24 h.[152] After group B, the commonest, these four are the most common groups causing meningococcal disease in the United States. Despite extensive research into production of a nonpolysaccharide vaccine or a polysaccharide conjugated with another immunogen, there is no commercially available group B vaccine at this time.

An improved meningococcal vaccine was developed using methods proven to be successful with conjugate polysaccharide vaccines for *Streptococcus pneumoniae*

and *Haemophilus influenzae*. As is true of these species, *N. meningitidis* capsular polysaccharides are T-cell-independent antigens and immunization does not elicit a memory response. This results in inducing a response that is neither long lasting nor characterized by anamnestic response after subsequent challenge with the same polysaccharide antigen. Thus meningococcal polysaccharide vaccines do not confer long-lasting immunity. Covalent coupling of polysaccharide to a protein carrier results in a conjugate vaccine and this changes the expression of the immune response to the polysaccharide from T-cell independent to T-cell dependent, resulting in an improved primary response to the polysaccharide and a strong anamnestic response with re-exposure. Proof that this method of vaccine design results in a potent infant vaccine has come from the experience with vaccines for *H. influenzae* type b and *S. pneumoniae*. Both conjugate *H influenzae* type b and conjugate *S pneumoniae* vaccines which were introduced for mass infant immunization in the United States in 1990 and 2000, respectively, and have been successful in reducing the incidence of disease caused by serotypes contained in the vaccines.[153,154] An added benefit of these conjugate vaccines is that they decrease the rate of nasopharyngeal colonization of the respective bacteria, thus providing unimmunized individuals protection through a herd-immunity effect. Conjugate meningococcal vaccine was introduced in the United Kingdom in 1999. With only immunogenicity and safety data three 3 monovalent serogroup C conjugate vaccines were introduced. Infants were given a 3-dose immunization series targeting all children between 12 months and 17 years of age. By 2001–2002, vaccine coverage in the United Kingdom was estimated at 80% in infants, 84% in toddlers, 76% in preschoolers, and 87% in school-aged children. Effectiveness of the meningococcal group C conjugate vaccines within the first year of immunization ranged from 88 to 98% in different age groups. Duration of the protective response is unknown.[40]

In the United States a quadrivalent conjugate meningococcal vaccine (Menactra, produced by Sanofi-Pasteur, Inc) was approved for individuals from 11 to 55 years of age in January 2005. Menactra contains the capsular polysaccharides from serogroups A, C, Y, and W-135 (4 μg each) conjugated to 48 μg of diphtheria toxoid (Tables 3 and 4). It is expected to provide longer-lasting protection from meningococcal disease than Menomune and may also be better at preventing the spread of disease from person-to-person. Protective concentrations of antibodies are achieved within 8 days of immunization. Both vaccines induce protective antibodies against serogroups in the vaccine in more than 90% of those who get the vaccine. Both prevent meningococcal disease due to serogroups A, C, Y, and W-135 but neither prevents disease due to meningococcal serogroup B.

For the polysaccharide vaccine antibody responses have been found to persist for at least 5 years.[155] There is insufficient data to recommend a revaccination schedule for the conjugate vaccine at this time.

9.3.1. Application. Before the development of the conjugated meningococcal vaccine the incidence of meningococcal disease in the US general population had been considered to be too low to warrant wide-scale immunization with the available meningococcal vaccines. In the United States, group A meningococcal infections have been very rare since the end of World War II, while groups B and C have provided most of the interim cases. In recent decades groups Y and W-135 have emerged as common where previously they had been rare.

On the other hand, the 4-year Brazilian epidemic[6] of the 1970s resulted in more than a quarter of a million cases. In the first 2 years, this was essentially a group C epidemic that then became a group A epidemic. A massive countrywide immunization campaign was undertaken, which eventually meant that more than 80 million persons received bivalent A and C, or monovalent A or C, vaccines. This

Table 3. Meningococcal Vaccines Available in the United States (2005)

Generic Name of Vaccines	Meningococcal conjugate vaccine	Meningococcal polysaccharide vaccine
Brand Name of Vaccine	Menactra	Menomune
Manufacturer	Sanofi Pasteur Inc.	Sanofi Pasteur Inc.
Year of Licensure	January 2005	1981
Recommended age of vaccines	2–55 years	> 2 years
Schedule of vaccination and route	Single dose, intramuscularly	Single dose, subcutaneously
Serogroups covered	A, C, Y, W-135	A, C, Y, W-135
Chemistry	Capsular polysaccharide linked to protein (diphtheria toxoid)	Capsular polysaccharide
Length of protection	Unknown	Excellent 3–5 years

Source: Meningococcal Disease and Meningococcal Vaccines April 2005. CDC, accessed at http://www.cdc.gov/nip/vaccine/mening/mening_fs.htm

Table 4. Meningococcal Vaccine is Recommended for:

- All children 11–12-year-olds at their routine preadolescent check-up
- If not previously vaccinated, adolescents should be vaccinated before they enter high school, at around 15 years of age
- People who have an elevated risk of meningococcal disease including:
  - ☐ College freshmen living in dormitories
  - ☐ Microbiologists who are routinely exposed to isolates of *N. meningitidis*
  - ☐ Military recruits
  - ☐ People who travel to, or live in countries where meningococcal disease is very common.
  - ☐ Anyone greater than 2 years of age whose spleen has been damaged or removed and persons with certain other immune system disorders.
  - ☐ Persons advised to receive vaccination during an outbreak

The following should not receive meningococcal conjugate vaccine:

- Anyone who has ever had a severe (life-threatening) allergic reaction to a previous dose of either meningococcal vaccine should not get another dose.
- Anyone who has a severe (life-threatening) allergy to any vaccine component should not get the vaccine.
- Anyone who is moderately or severely ill at the time the shot is scheduled should probably wait until they recover. People with a mild illness can usually get the vaccine.
- Anyone who has had Guillien Barré syndrome.
- Meningococcal vaccines may be given to pregnant women. However, MCV-4 is a new vaccine and has not been studied in pregnant women as much as MPSV-4 has.

brought the epidemic to a close and it did not recur. In Finland, a large outbreak in the military, accompanied by many civilian cases, was also treated with vaccine and was soon brought under control.[7]

As a result of the successes of the conjugate polysaccharide vaccines for *H. influenzae* and *S. pneumoniae* and recognition of the relatively high risk of older adolescents for meningococcal infection, recommendations for meningococcal vaccine have recently been revised.[156] Table 4 shows the current recommendation for meningococcal vaccine. Menactra is recommended as the preferred vaccine for those 2–55 years of age. For others greater than 2 years old, Menomune is recommended. Vaccination is indicated where localized or institutional outbreaks can be defined and the causative strain identified as either group A, C, Y, or W-135. A guideline has been provided by the Centers for Disease Control and Prevention. Meningococcal vaccine can be considered for general use in a defined population at risk if there are three or more primary cases with the same serogroup and the serogroup-specific rate of meningococcal infection in a defined population exceeds 10 times the baseline or expected rate for that serogroup over a 3-month period. (generally $\geq$10/100,000 population) Usage to prevent secondary cases among close contacts is recommended by the CDC,[157] but the overall risk is low and most such cases occur within less than 6 or at most 9–10 days of the index case. Thus, chemotherapeutic measures should be more effective.

In addition, patients with functional or anatomic asplenia, such as in sickle-cell disease or traumatic rupture of the spleen, are prone to severe or fatal infections with polysaccharide-encapsulated bacteria. Individuals with deficiencies of the terminal complement components may also have recurrent meningococcal infections. Patients over 2 years of age who fit any of these categories should receive the current meningococcal vaccine as part of their preventive care. While younger patients might have benefit from the serogroup A component, the risk of group A disease is too low to warrant vaccine in most Western countries.

Meningococcal vaccine is also recommended for travelers to countries known to have an epidemic of meningococcal disease or where the endemic rate is very high such as sub-Saharan Africa or in certain regions of Saudi Arabia at the time of the *Hajj*.

10. Unresolved Problems

The greatest unresolved problems are the elucidation of the factors that lead to the occurrence of a case; i.e., of the many individuals who acquire meningococci carriage, why do only so few (probably less than 10%) develop meningococcal disease? In parallel with this is the question of which factor or factors lead to the occurrence of an epidemic in a given locality or institution. Such epidemics arise suddenly and for no apparent reason persist for varying periods of time—days, weeks, months, or years—and then either disappear completely or are replaced by meningococci of another serogroup. Are the factors responsible for these fluxes changes in the pathogen, the host or a combination of the two?

Questions remain concerning the use of the current meningococcal vaccines. This involves questions of optimal timing and the definition of the population that would benefit most from their use, as well as the definition of the conditions under which they achieve maximum effectiveness. There are few data that address the question of revaccination. We still lack vaccines for children under 2 years of age that will protect against the major serogroups. The new conjugate meningococcal vaccine may be useful for these children but only limited studies in infants and young children are available. We still lack an effective vaccine for group B *N. meningitidis*.

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12. Suggested Reading

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CHAPTER 25

Mycoplasma pneumoniae and Other Human Mycoplasmas

Deborah F. Talkington and Ken B. Waites

1. Introduction

Mycoplasmas are a class of fascinating wall-less bacteria that cause disease in humans, animals, and plants. They include species recognized as among the smallest known free-living cells. These microbes have very small genomes and unique genetic features. Their temporary classification as viruses and fastidious growth requirements set the stage early for the use of diagnostic techniques better suited to viral detection and for the well-known difficulties of diagnosing infections in the clinical laboratory. Systemic infections are well known in both immunocompetent and immunocompromised patients, but the host and bacterial factors that allow invasion from epithelial colonization sites are poorly understood. The focus of this chapter will be respiratory and extrapulmonary infections caused by *Mycoplasma pneumoniae* and genitourinary tract infections caused by *M. hominis*, *M. genitalium*, and *Ureaplasma* species. Some less common mycoplasma infections will also be addressed.

2. Historical Background

In 1898, Nocard and Roux reported the first culture isolation of a mycoplasma, the bovine pleuropneumonia agent now known as *M. mycoides* subsp. mycoides.[1] Evidence gradually accumulated that mycoplasmas had a role in various diseases of animals and possibly in human disease. Klieneberger introduced the concept in the 1930s that mycoplasmas were "L forms" of bacteria lacking cell walls and living symbiotically with other walled bacteria.[2] This theory started a spirited debate pitting those who believed that mycoplasmas were unique species against those who believed that mycoplasmas were wall-less variants of other known bacterial species and were undeserving of a unique taxonomic representation (this controversy was not completely settled until the 1960s when DNA analysis showed that mycoplasmas were indeed unique bacteria that lacked the ability to produce cell walls under any circumstances). Dienes and Edsall are commonly credited with the first mycoplasma isolation from humans in 1937 when they reported what is believed to be *M. hominis* in culture from a Bartholin gland abscess.[3–6]

In 1944, Eaton succeeded in isolating a "filterable" agent from the sputum of a patient with primary atypical pneumonia using tissue culture.[7] Tests on volunteers and field studies conducted in the 1950s and 1960s provided definitive evidence that the "Eaton agent" caused lower respiratory tract infections in humans.[8,9] The Eaton agent was considered to be a virus until it became clear that antibiotics could be effective against it, and in 1961 Marmion and Goodburn postulated that the Eaton agent was a PPLO.[10] Culture in cell-free medium was achieved and in 1963 the taxonomic designation *M. pneumoniae* was proposed.[11]

The term "mycoplasma" (Greek; "mykes" = fungus and plasma = "formed") emerged in the late 1950s as a less linguistically challenging replacement for the term "pleuropneumonia-like organisms," or "PPLO." The allusion

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Table 1. Selected Mycoplasmas Cultured from Humans[a]

Organism	Primary Site of Colonization		Known Disease Role in Immunocompetent People?
	Respiratory Tract	Genitourinary Tract	
Acholeplasma laidlawii	+	–	No
Mycoplasma buccale	+	–	No
Mycoplasma faucium	+	–	No
Mycoplasma fermentans	+	+	Yes?
Mycoplasma hominis	–	+	Yes
Mycoplasma genitalium	–	+	Yes
Mycoplasma lipophilum	+	–	No
Mycoplasma orale	+	–	No
Mycoplasma pirum	?	?	No
Mycoplasma penetrans	–	+	?
Mycoplasma primatum	+	+	No
Mycoplasma salivarium	+	–	No
Mycoplasma pneumoniae	+	–	Yes
Mycoplasma spermatophilum	–	+	No
Ureaplasma urealyticum	–	+	Yes
Ureaplasma parvum	–	+	Yes
Mycoplasma amphoriforme	+	?	?

[a]Adapted from[5]

to a fungus-like growth pattern in the name happens to describe only the growth of *M. mycoides*, but the term was nevertheless adopted. As the single order of mycoplasmas grew to three orders by the late 1960s, mycoplasmas were elevated to a class named Mollicutes, which derives from Latin words meaning soft ("mollis") and skin ("cutis"). The term Mollicutes is now used in general to refer to the current four orders.[4,6]

Of more than 150 mycoplasma species identified, fewer than 20 are known to colonize or infect immunocompetent humans, with most showing a predilection for either the respiratory or urogenital tracts in normal individuals (Table 1).

3. Methodology

3.1. Clinical Diagnosis

The clinical presentation of *M. pneumoniae* respiratory disease is similar to that seen with other atypical bacterial pathogens such as *Chlamydophila pneumoniae* and legionellae and in some cases infections with *Streptococcus pneumoniae* and various respiratory viruses and rickettsiae. *M. pneumoniae* may be present in the respiratory tract concomitantly with other pathogens,[12,13] and a chronic carrier state may be apparent in the absence of overt disease.[14]

The radiographic findings in mycoplasmal pneumonia can be extremely variable and mimic a wide variety of lung diseases, but sometimes the radiologic appearance coupled with clinical manifestations give clues to a mycoplasmal etiology. The inflammatory response elicited by *M. pneumoniae* causes interstitial mononuclear inflammation in the lungs that may be manifested radiographically as bronchopneumonia of the perihilar regions or lower lobes, usually with a unilateral distribution, and hilar adenopathy. However, lobar consolidation has been described, and the degree of consolidation may exceed what would be expected based on the severity of clinical manifestations. Among patients with mycoplasma pneumonia who require hospitalization, over 10% will require mechanical ventilation.[15] Pleural effusions and diffuse alveolar damage sometimes occur in association with more severe cases,[16–18] and long-term sequelae such as bronchiectasis and pulmonary fibrosis have been reported.[19,20] In some patients, the organism may persist for weeks to months in the lower respiratory tract following acute pneumonia, resulting in prolonged reduced pulmonary clearance and airway hyper-responsiveness.[21] Mycoplasmal pneumonia can be very severe in otherwise healthy people.

Although a few case reports of *M. pneumoniae* infections in pediatric AIDS patients have appeared in the literature[22] and there has been speculation about a possible role for *M. pneumoniae* as a cofactor in AIDS,[23,24] it is not known if the incidence or severity of pulmonary or extrapulmonary infections in AIDS patients is increased. Hypogammaglobulinemic patients appear to be at increased risk of developing joint and respiratory infections,[24–27] but the overall effect of the immunosuppressed state on host resistance to infections is unclear. There have been reports of transplant recipients becoming susceptible to infection with other species of

mycoplasmas in a variety of sites, and because *M. pneumoniae* may not be killed in donor tissues by antibiotics, there is the possibility that infection could be acquired by transplant recipients on immunosuppressive drugs (reviewed in[28]).

3.2. Laboratory Diagnosis

3.2.1. Clinical Laboratory Diagnosis. Clinical laboratory findings are seldom diagnostic. About one-fourth to one-third of persons with lower respiratory tract infections due to *M. pneumoniae* will have leukocytosis and/or elevated erythrocyte sedimentation rate. Sputum Gram stain may show mononuclear cells or neutrophils and normal flora. There are no hepatic or renal abnormalities typical of mycoplasmal infection, although some patients may develop a hemolytic anemia as one of the numerous extrapulmonary manifestations, and this may be reflected in the hemogram.

3.2.1.1. Cold Agglutinins. Prior to the widespread availability of commercialized antibody assays, and even before the precise bacteriological characterization of the etiological agent was known, clinicians sometimes used the presence of cold agglutinins to confirm their clinical suspicions of primary atypical pneumonia, also known as "cold agglutinin disease". Cold agglutinins are IgM antibodies produced 1–2 weeks after initial infection in about 50% of *M. pneumoniae* infections, sometimes persisting for several weeks. The exact antibodies responsible for cold agglutination are not well characterized; they may be the result of cross-reactive autoantibodies developed against the I antigen of human erythrocytes during acute mycoplasmal infection. Another possibility is that antibodies are directed against erythrocyte antigens altered during infection. The latter possibility is supported by the fact that *M. pneumoniae* is known to interact with and adsorb to human erythrocytes and to produce a hemolysin, and also because cold agglutinins can be induced in rabbits inoculated with human erythrocytes that were preincubated with *M. pneumoniae*, but not in rabbits inoculated with either *M. pneumoniae* or erythrocytes alone.[5]

Two varieties of cold agglutinin determination have been used. The simplest version is referred to as "bedside cold agglutinins." Blood from a patient suspected of having mycoplasmal pneumonia is collected into a tube containing citrate or another suitable anticoagulant, then placed in ice water in a freezer for approximately 30s. The tube is then tilted on its side and examined for coarse agglutination. On warming, the agglutination should resolve, but it can be reproduced by repeating the cooling procedure. More precise testing can be performed using doubling dilutions of patient sera with human type O erythrocytes. The cold agglutinin response often correlates directly with the severity of pulmonary involvement, and patients with extensive lobar involvement will usually have a titer of $\geq$32. In contrast, those with only minimal illness may not develop cold agglutinins.

Cold agglutinins also occur in persons who have various bacterial and rickettsial infections as well as in those with influenza virus and adenovirus infections. Due to the availability of antibody assays that are based on immunological reagents specific for *M. pneumoniae*, the popularity of testing serum for the presence of cold agglutinins has declined. If used, this test should be limited to persons in whom mycoplasmal pneumonia is a strong possibility. Under these circumstances, a positive test provides reasonable supportive evidence of a mycoplasmal etiology, on the basis of which clinical management decisions can be made.[5,29]

3.2.1.2. Culture. Culture of *M. pneumoniae* from the respiratory tract and other body sites is laborious and expensive, requiring serial blind passages, specialized and expensive growth media, and incubation periods of up to several weeks. SP4 medium ("SP" for "spiroplasma"), developed over 20 years ago by Tully et al.,[30] has become the most successful and widely used broth and agar medium for cultivating *M. pneumoniae* for clinical purposes. It is sold commercially in the United States by Remel Laboratories (Lenexa, Kansas). Detection of *M. pneumoniae* by culture is predicated on its characteristic hydrolysis of glucose, with a resultant acidic shift, after 4 days or more of incubation in broth containing a phenol red pH indicator. Initial specimens, as well as broths with color change and blind subcultures, should be transferred to SP4 agar, incubated, and examined under a stereomicroscope at regular intervals to look for development of spherical colonies of up to 100 μm in diameter (Figure 1).

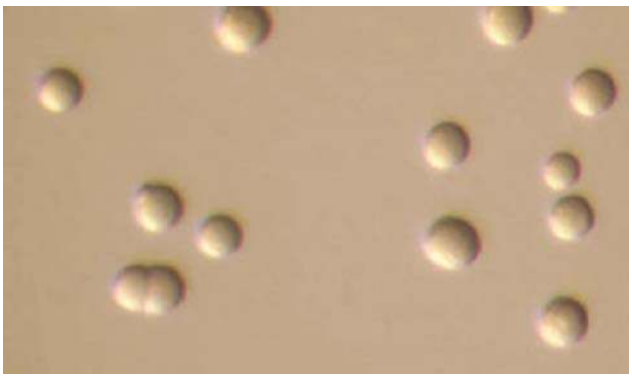


Figure 1. Spherical colonies of *M. pneumoniae* growing on SP4 agar at a magnification of ×95. Reprinted with permission from authors and American Society for Microbiology.[5]

Incubation of *M. pneumoniae* broth cultures under atmospheric conditions at 37°C is satisfactory, but agar plates will yield the best colonial growth if 5% CO_2 is provided and plates are sealed to prevent loss of moisture during prolonged incubation. A biphasic medium consisting of an agar slant in a screw-cap bottle to which broth is added is sold by Irvine Scientific in the United States under the trade name Mycotrim RS.[5,31,32]

Compared to molecular techniques such as PCR, the analytic sensitivity of culture may be no more than 60% in experienced laboratories, even when rigorous adherence to procedures known to enhance cultural isolation are used.[33] However, when positive, culture has the advantage of being 100% specific, provided that appropriate additional procedures are used to identify the organism isolated to species level. Although there is good evidence that disease is associated with increased numbers of bacteria in the upper respiratory tract,[34] the persistence of *M. pneumoniae* for variable lengths of time following acute infection makes it difficult in some cases to assess the significance of a positive culture or assay without additional confirmatory tests such as serology. For the reasons cited above, culture is rarely used for routine diagnosis and/or management of *M. pneumoniae* infections. In view of the many limitations of culture for detection of *M. pneumoniae*, if attempted it should be augmented by additional diagnostic methods such as PCR and/or serology.

3.2.1.3. Serology. Commercial *M. pneumoniae* antibody detection kits which utilize passive (particle) agglutination, indirect immunofluorescence, or enzyme-linked immunofluorescent assay (EIA) formats are available for the detection of antibodies to *M. pneumoniae* in human sera (reviewed in[31]).

The strategy for serologic diagnosis of *M. pneumoniae* was strongly influenced by early assumptions that mycoplasmas were viruses. This led to the development of complement fixation (CF) tests for *M. pneumoniae* diagnosis. The sensitivity and specificity of CF tests for viral infections are due in part to the relatively low number of viral antigens, which reduces the chances of cross-reactivities. Because of the much larger complement of bacterial antigens, the CF test for *M. pneumoniae* suffers from decreased sensitivity and specificity, limiting its usefulness in serologic testing,[4,31,32,35] and with the wide variability in performing and interpreting CF assays between laboratories, CF is no longer widely utilized. Most laboratories have replaced the CF assay with EIAs due to their ease of use and decreased run time. Of the various test formats, EIA is the most widely used and the most adaptable to most clinical laboratory settings.[35–37]

EIA is a valid methodology for detecting IgM antibodies to *M. pneumoniae* using acute-phase serum from some patients with suspected cases of *M. pneumoniae* infections.[38–40] This approach has a theoretical advantage because only one specimen is required, and IgM can be detected approximately 7–10 days after the onset of infection. However, a major limitation of an IgM-specific test is that detectable levels of IgM antibodies may not be present if the serum sample is obtained too early in the infection, and only 14–27% of acute-phase sera from confirmed adult patients may test positive by the various IgM kits. The number of IgM-positive sera from confirmed cases rises to 39–88% when convalescent-phase sera are tested.[36]

Alternately, specific IgM in adults does not always indicate an acute infection because it can persist for up to a year after infection with *M. pneumoniae*.[41,42] To further complicate matters, an IgM response may be either minimal or undetectable when adults are reinfected. In previous studies, approximately 20% of adults did not mount an IgM response after infection with *M. pneumoniae*.[35,43] Therefore, relying exclusively on the detection of specific IgM (especially in an adult population) will result in the misdiagnosis of some infections. Measurement of IgA, which may rise and fall sooner than IgM or IgG, can be useful for detection of acute *M. pneumoniae* infection, especially in older persons, but reagents to detect this antibody are available in very few commercial tests at the present time. A comprehensive review of the value of serology for the detection of *M. pneumoniae* in the clinical laboratory has been published by Waites et al.[32]

3.2.1.4. Antigen Detection. Rapid assays for detection of (non-nucleic acid) *M. pneumoniae* antigen in respiratory tract specimens have included direct immunofluorescence, counterimmunoelectrophoresis, immunoblotting, and antigen capture enzyme immunoassay. The utility and general acceptance of these techniques have been reduced by low sensitivity and cross-reactivity with other mycoplasmas found in the respiratory tract. Non-amplified antigen detection assays are at the limit of sensitivity for detection and are not recommended for diagnostic purposes when superior technology utilizing nucleic acid amplification is available. Although not sold in the United States at present, commercial antigen detection tests for *M. pneumoniae* are available in some European countries.[44–46]

3.2.1.5. Nucleic Acid Detection. Development of molecular-based testing such as the polymerase chain reaction (PCR) assay has lessened the importance of culture as a means for detecting *M. pneumoniae*. Studies since the late 1980s using simulated clinical specimens, animal models, and later clinical trials have validated the ability of PCR to detect *M. pneumoniae*, often in conjunction with serology and/or culture (recently reviewed in[5]). The same types of clinical specimens that can undergo culture can also be tested

by PCR. The use of two different targets can maximize the ability to detect the organism. The conventional PCR procedure currently used at the Centers for Disease Control and Prevention has been adapted from the procedure originally described by Bernet et al.[47] using primers derived from the *M. pneumoniae* ATPase gene. They have also developed a real-time PCR which targets this gene. Other sequences, primarily the P1 adhesin and conserved regions of 16S rRNA, have also been utilized. For additional information and tabulations of various nucleic acid amplification assays according to respiratory specimen tested, or by publication with or without validation data, the reader is referred to three recent publications on this topic.[48–50] Among the other advantages of PCR are that it can be used to detect mycoplasmas in tissue that has already been processed for histological examination or in cultures that are contaminated. PCR requires only one specimen, the assay can be completed in 1 day, it may provide a diagnosis earlier in infection than serology, and it does not require viable organisms. Specific advantages of RNA-based amplification techniques are the high sensitivity that can be achieved due to the large number of rRNA copies per mycoplasmal cell and the fact that its detection may be more indicative of viable mycoplasmas in a clinical sample.[48]

3.3. In Vitro Antibiotic Susceptibility Testing

M. pneumoniae is inhibited by tetracyclines, macrolides, ketolides, and fluoroquinolones, with little variation in MICs among clinical isolates.[51,52] Thus, in vitro susceptibility testing is not indicated for routine patient management purposes for infections of the respiratory tract. Additional agents that are active at the bacterial ribosome, such as streptogramins, chloramphenicol, and aminoglycosides, may also show in vitro inhibitory activity against *M. pneumoniae* but are not widely used for therapeutic purposes against this organism. Clindamycin may be effective in vitro, but limited reports suggest that it may not be active in vivo, and it has not been considered a first-line treatment.[53] Due to the lack of a cell wall, all mycoplasmas are innately resistant to all beta-lactams and glycopeptides. Sulfonamides, trimethoprim, polymyxins, nalidixic acid, and rifampin are also inactive. Linezolid is the prototype agent of the oxazolidinone class. These agents are much less active against *M. pneumoniae* than the other agents that inhibit protein synthesis.[54]

New quinolones such as moxifloxacin, gemifloxacin, and sparfloxacin tend to have somewhat greater in vitro activity than older agents such as ciprofloxacin, ofloxacin, and levofloxacin, although MICs of all fluoroquinolones are several-fold higher than those of macrolides.[51,52,54,55] Fluoroquinolones have been shown to be bactericidal for *M. pneumoniae*, whereas macrolides and tetracyclines are primarily bacteriostatic.[51,55–57]

The extent of naturally occurring, clinically significant acquired antimicrobial resistance of *M. pneumoniae* to any drug class is not known, but historically has been believed to be uncommon. Strains resistant to tetracycline and erythromycin have been isolated but are still believed to be uncommon in the United States, even though there has been a recent increase in macrolide resistance in Japan since 2000 due to 23s rRNA mutations which may have some clinical significance.[5] Recent studies evaluating macrolides, tetracyclines, or fluoroquinolones indicate comparable in vitro activities against isolates obtained from North America and Europe, with no change from data reported earlier other than some minor variations that may be due to differences in testing methods, media, and inocula, although some macrolide-resistant isolates from the United States and France are known to occur.[51,58–60] Since organisms from acute *M. pneumoniae* infections are seldom sought by culture, they are almost never tested for in vitro susceptibilities for clinical purposes, and most in vitro studies reported to date tested relatively small numbers of clinical isolates from limited geographic areas. Older studies reported that organisms can be shed in respiratory secretions for long periods after acute infection and that high-level, macrolide-resistant strains can be isolated following erythromycin treatment.[61,62]

4. Etiologic Patterns

4.1. *M. pneumoniae* Syndromes

It is important to realize that *M. pneumoniae* infection can present as a very wide variety of clinical manifestations. A brief summary is provided in Table 2.

4.1.1. Pneumonia. The radiographic findings in *M. pneumoniae* pneumonia can be extremely variable and mimic a wide variety of lung diseases, but sometimes the radiologic appearance coupled with clinical manifestations can give clues to a mycoplasmal etiology. The inflammatory response elicited by *M. pneumoniae* causes interstitial mononuclear inflammation in the lungs that may be manifest radiographically as bronchopneumonia.[63]

4.1.2. Asthma. Multiple reports suggest that *M. pneumoniae* infections are associated exacerbations of asthma,[29,64–67] although this finding is not universal.[65,68] The more important question is whether mycoplasmas are a primary cause of asthma or if mycoplasma infection is a cofactor in asthma development. Multiple lines of evidence suggest that *M. pneumoniae* may play a role beyond simple and acute exacerbation. *M. pneumoniae* can be recovered

Table 2. Manifestations of *Mycoplasma pneumoniae* Infection

Respiratory	**Common secondary**
Pneumonitis/pneumonia	Erythematous maculopapular, other rashes
Bronchitis, tracheobronchitis	Thrombocytopenia
Bronchiolitis	Meningitis – encephalitis
Tracheobronchitis	Mild anemia
Pharyngitis	Asthmatic bronchitis
	Otitis externa, interna
Neurological	**Less common secondary**
Meningitis	Hemolytic anemia
Encephalitis	Disseminated intravascular coagulation
Psychosis	Thromboembolism
Guillain–Barré syndrome	Pericarditis, myocarditis, pericardial effusion
Cerebellar ataxia	Glomerulonephritis, renal failure
Brain-stem syndrome	Arthritis, polyarticular and septic
Poliomyelitis-like syndrome	Erythema nodosum
Transverse myelitis	Stevens–Johnson syndrome
Focal encephalopathy	Conjunctivitis

more often from the airways of patients with chronic, stable asthma than from matched control patients,[69–71] and reductions in lung function tests 1 year,[72] 3 years,[73] and up to 9 years or more[74] after an episode of mycoplasma respiratory tract infections have been reported, thus establishing the ability of mycoplasmas to induce chronic to permanent lung damage long after resolution of respiratory tract symptoms. Similarly, bronchial hyperreactivity to methacholine challenge was present in 50% of patients 12 weeks after recovering from mycoplasma pneumonia compared with none in controls.[75] *M. pneumoniae* is known to induce a number of inflammatory mediators implicated in the pathogenesis of asthma. A variety of mediators (including IgE) are induced at high levels by mycoplasma infection and may play a role in exacerbations, which often include wheezing.[71,76,77]

Although a delay in initiation of proper antibiotic therapy may be a risk factor for potentially permanent reductions in lung function,[72] conflicting reports in the literature suggest that much more research is needed to determine the true role of *M. pneumoniae* in the initiation and chronicity of asthma.

4.1.3. Extrapulmonary Syndromes. Reports of extrapulmonary *M. pneumoniae* complications have appeared in the literature for over 60 years. Although the complications can be grouped roughly by organ system for ease of discussion, it is important to realize that often multiple organ systems are involved, and hematogenous spread to many organ systems in the same patient is well known.[21,78,79] It is very important to realize that extrapulmonary complications can be seen before, during, or after pulmonary manifestations or even occur in the complete absence of any respiratory symptoms.[80]

4.1.3.1. Neurologic. *M. pneumoniae* CNS infections can be severe and life threatening and are more common than generally perceived. In a study of 462 children with acute encephalitis, patients with *M. pneumoniae* infection were seven times more likely to die or have severe neurologic sequelae than were other children in the cohort,second only to herpes simplex virus.[81] In a 5-year prospective study of 159 children with encephalitis, 20 (13%) were characterized as probable or possible cases of *M. pneumoniae*, but only 13 of the 20 (65%) had prodromal respiratory signs and symptoms, which ranged from 4 weeks to several days prior to the onset of clinical encephalitis.[82] Others have found that the incidence of prior respiratory illness in children diagnosed with *M. pneumoniae* CNS infections ranges from 20 to 80%.[83] These findings emphasize that the absence of respiratory illness prior to the onset of encephalitis does not rule out involvement by *M. pneumoniae*.

In children, most CNS infections attributed to *M. pneumoniae* manifest as encephalitis (diffuse, local, or expansive). However, a wide range of disease syndromes in adults and/or children have been reported, including encephalitis, post-infectious encephalomyelitis with demyelination, acute disseminated encephalomyelitis, meningoencephalitis, meningitis, transverse and diffuse myelitis, Guillain–Barré syndrome, Bickerstaff's brain-stem encephalitis, Fisher's syndrome, and cranial (as well as peripheral) nerve neuropathy, including optic neuritis. In addition, cerebellar syndrome, coma (with or without evidence of meningoencephalitis), stupor, ataxia, athetosis, psychosis, diplopia, mental confusion, transient extreme spindles (secondary to subacute encephalitis), status epilepticus, grand mal seizure, and athetotic/choreoathetotic movements have been reported as concurrent manifestations or sequelae.[84–87]

M. pneumoniae infection can induce antibodies against the galactocerebroside (Gal-C) component of myelin in 25% of patients without CNS involvement and in 100% of patients with such involvement. High anti-Gal-C antibody titers have been reported in cases of profound spinal cord degeneration and decerebrate rigidity and coma subsequent to mycoplasma respiratory tract infections.[88] The mechanism by which anti-Gal-C antibodies are induced by *M. pneumoniae* infection is not known, but the integrity of the blood–brain barrier may be a determining factor in the extent of damage to CNS structures. Alternately, such antibodies may also be induced after CSF invasion, as demonstrated by

the presence of anti-mycoplasma antibodies in the CSF of patients with mycoplasmal infection of the CSF (as demonstrated by culture or PCR).[89]

Numerous reports have documented the presence of *M. pneumoniae* in CSF and/or brain tissue, proving that invasion may occur (reviewed in[6]). However, CNS disease associated with *M. pneumoniae* infection may occur without evidence of CNS invasion, and faced with clear evidence that *M. pneumoniae* can induce anti-myelin antibodies, researchers have postulated that an immune-mediated mechanism (in addition to direct invasion) is involved in CNS disease. Narita et al. expanded on this concept in several reports when they showed that *M. pneumoniae* was detectable by PCR in the CSF of patients more frequently when the onset of CNS symptoms appeared within 7 days of the onset of fever versus 8 or more days.[90–92] They interpreted this temporal data to suggest that two distinct patterns of CNS disease caused by *M. pneumoniae* exist: an early-onset syndrome caused by the presence of bacteria in the CNS and a late-onset syndrome in which the presence of bacteria in the CNS is not necessary to cause disease. Reports of post-infectious leukoencephalopathy with severe sequelae also support a role of autoimmunity in mycoplasmal CNS disease.[93] However, findings by other workers do not neatly fit into this two-pattern model, and much more work is required to determine its validity and usefulness.[94]

Neurologic complications from respiratory *M. pneumoniae* infection may ultimately result from direct infection of brain tissues as well as secondary destruction of tissue caused by the generation of autoimmune antibodies, but only recently have assays evolved to reliably identify low numbers of mycoplasmas in tissues so that this issue can be properly addressed.

In a multi-year CDC program designed to uncover infectious disease diagnoses for patients with unexplained serious illnesses in the United States, *M. pneumoniae* was responsible for cases characterized by primarily neurologic symptoms as well as those with primary respiratory symptoms.[95] For a more detailed review of neurologic diseases caused by *M. pneumoniae*, see reference[6].

4.1.3.2. Cardiac. A variety of cardiac complications have since been reported, including pericarditis, myocarditis, and pericardial effusion with and without cardiac tamponade.[22,25,96–98] Of note, a prospective study of pericardial effusions uncovered two cases of *M. pneumoniae* infection confirmed by isolation of mycoplasmas from pericardial effusion or tissue. An additional two cases of pericarditis due to *M. hominis* (and one due to *Ureaplasma urealyticum*) have also been diagnosed.[99]

In general, cardiac complications are relatively uncommon, occurring in 1–8.5% of patients with serologic evidence of infection, primarily adults.[13] A large 1979 study reported that 4.5% of patients with serologically confirmed cases of mycoplasmal pneumonia had cardiac complications, and almost half of these patients had evidence of symptoms or heart abnormalities an average of 16 months later.[98]

4.1.3.3. Renal. Renal complications associated with *M. pneumoniae* respiratory infections were first reported in the 1970s. Renal diseases such as acute glomerulonephritis and renal failure (sometimes secondary to rhabdomyolysis), tubulointerstitial nephritis, IgA nephropathy, as well as others have been reported sporadically.[79,99–102] Attempts to demonstrate mycoplasma antigen in damaged renal tissue with immunohistochemical techniques have not been uniformly successful, once again leading to theories that an antibody-mediated pathogenesis and not direct infection may be responsible for some cases of renal disease.[100,103] An attempt to use PCR to identify mycoplasmas in renal tissue from four young patients with acute nephritis with serologic evidence of recent *M. pneumoniae* was unsuccessful.[21] Although renal complications of *M. pneumoniae* infection are rare,[21] additional attempts to identify mycoplasmas in kidney tissue using PCR assays are needed to clarify whether direct or indirect damage to tissue is occurring.

4.1.3.4. Arthropathies. Three basic patterns of joint disease associated with *M. pneumoniae* infection have been described: transient polyarticular pain or discomfort centered primarily on the large joints coincident with acute illness, polyarthropathy affecting primarily medium-sized joints that can persist for a year or more after acute illness,[104] and septic arthritis reported most often in hypogammaglobulinemic patients.[26,27,105107] However, in only a few cases had mycoplasmas been cultured from joint fluid until the mid- to late 1980s, when reports of *M. pneumoniae* isolations from joint fluid from immunocompetent patients with polyarthritis appeared.[108] The difficulties of isolating mycoplasmas from affected joints of patients who fit clinical criteria for mycoplasma pneumonia invite suggestions that *M. pneumoniae* and other mycoplasmas may be "cofactors" in arthropathies such that direct invasion of joints may not be necessary.[109] Increased use of PCR assays to detect mycoplasmas from joint fluid and tissues will better establish how often mycoplasmas are really present in joints, but not cultivable. This will help clarify the role of direct invasion.

4.1.3.5. Dermatologic. A variety of dermatologic abnormalities have been associated with *M. pneumoniae* infection in up to 25% of patients.[26] Among the more

common are erythematous maculopapular and vesicular rashes or "exanthems." Most cases associated with pneumonia are self-limiting within about 7 days, and for unknown reasons are most often seen in males.[80] More severe mucocutaneous syndromes have also been reported, including severe conjunctivitis, generalized ulcerative stomatitis, and bullous exanthems.[80,110] Mild to severe forms of Stevens–Johnson syndrome have also been associated with *M. pneumoniae* infections, most often presenting as cases of erythema on the trunk with stomatitis and sometimes conjunctivitis rather than the severe toxic reactions with widespread epidermal necrosis more commonly seen with drug reactions.[111–113] The value of using steroids to treat Stevens–Johnson syndrome caused by *M. pneumoniae* has not been clearly established.[111]

Pediatric patients with pleuropneumonia are more likely to present with dermatologic manifestations than are those with uncomplicated pneumonia.[114] Clinicians should keep in mind that the presence of erythematous maculopapular rashes can also be caused by a number of antibiotics commonly given to *M. pneumoniae* patients.[115]

4.1.3.6. Hematologic. Hemolytic anemia has been recognized as a rare but severe complication of mycoplasma pneumonia since the 1940s.[116] Numerous reports have documented this complication, found in higher incidence among children.[117–119] Hemolytic anemia has been attributed in cold agglutinin disease and autoimmune hemolytic anemia to *M. pneumoniae*-induced cross-reacting antibodies to the I antigen of human erythrocytes.[53,118,120]

One report suggests that thrombotic thrombocytopenic purpura (TTP) sometimes seen in patients with *M. pneumoniae* infection may be the result of cross-reactive antibodies inactivating the plasma von Willebrand factor cleaving protease, resulting in unusually large von Willebrand factor multimers that later on initiate the cascade of TTP.[121] Pediatric cases of aplastic anemia associated with *M. pneumoniae* also have been reported.[122] When subclinical forms of hemolytic anemia and intravascular coagulation are considered, over 50% of patients with mycoplasma infections may be affected.[123]

4.1.3.7. Other Extrapulmonary Syndromes. A variety of less common extrapulmonary infections and sequelae have been reported for *M. pneumoniae*. Although rare, ocular manifestations have been reported, primarily in pediatric cases. Ocular manifestations include conjunctivitis, anterior uveitis, optic neuropathy, retinitis and retinal hemorrhages, iritis, and optic disc swelling, with or without permanent degradation of vision.[124,125]

Up to one-third of patients may have nonspecific ear symptoms, in addition to otitis externa and interna.[112,126] Rarely, hepatitis and pancreatitis have been associated with respiratory infections,[119,127] and *M. pneumoniae* has been isolated from the urogenital tract of males and females as well as cultured from a tubo-ovarian abscess.[128]

5. Descriptive Epidemiology

Epidemics of *M. pneumoniae* infections occur in the community and in closed or semi-closed settings. Military bases,[12,129–132] hospitals,[133–135] religious communities,[136,137] and facilities for the mentally or developmentally disabled have been particularly hard-hit.[138–140] Unlike endemic disease which has no seasonality, outbreaks in the United States tend to occur in the summer or early fall.[12,123,138,139,141]

New recruits on military bases are at particularly high risk of acquiring *M. pneumoniae* infection.[130] In the 1970s, more than half of all recruits acquired an acute mycoplasma infection in some companies.[129] Although the majority of infections are mild, *M. pneumoniae* is a leading cause of bacterial pneumonia among both outpatient and hospitalized military personnel.[131,142]

Outbreaks in closed or semi-closed settings can be very difficult to control for many reasons, including delayed outbreak detection, difficulty of implementing effective infection control practices, and a long incubation period. Attack rates can be quite high, with reports ranging from 25 to 71% in some settings.[12,139,141,143] Figure 2 shows successive peaks of illness in students at a military academy after they returned to school in two major groups. Although long-term morbidity is uncommon, these outbreaks can be very disruptive and can consume large resources. Strategies to control these outbreaks have included cohorting and using antibiotic therapy for symptomatic persons. A number of trials of prophylactic antibiotics have been tried with varied success. Many of these studies were longitudinal in nature, making it difficult to determine whether the interventions were effective or whether all susceptible persons had been exhausted. In one recent study, a placebo-controlled trial of azithromycin prophylaxis in a hospital for developmentally impaired adults, therapy demonstrated a protective efficacy of 75% against illness resulting in physician visits among hospital employees.[138] In selected circumstances, the use of prophylactic antibiotics may be an effective tool for control of mycoplasma outbreaks in closed settings.

5.1. Geographic Distribution

Infection with *M. pneumoniae* is worldwide, although careful survey studies have not been performed in most countries.

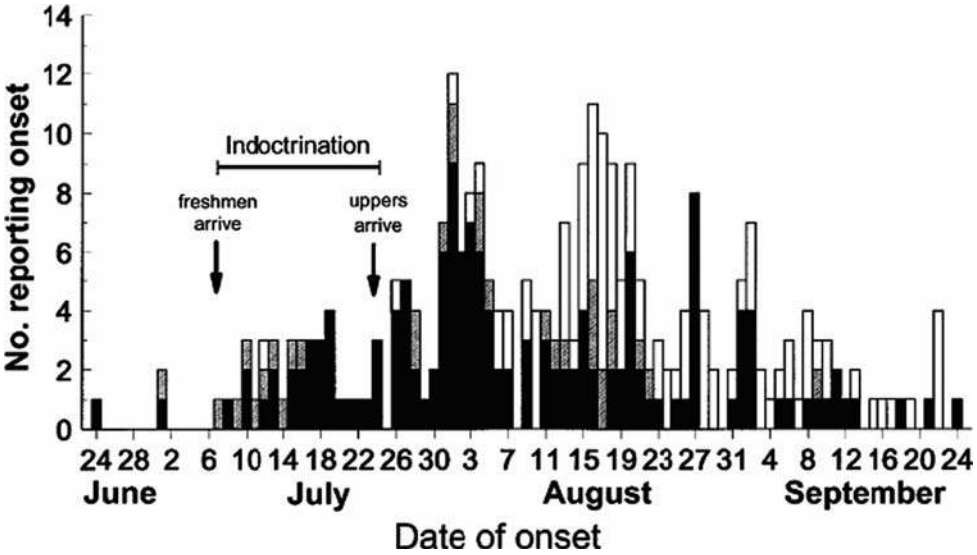


Figure 2. Outbreak of "mycoplasma-like" illness at a federal training academy. Shown is date of onset of illness among students presenting to clinic with condition (n = 273) during an outbreak at a federal service training academy, June–September 1996. Black bars, freshman; grey bars, upperclassmen (uppers) at indoctrination; white bars, remaining upperclassmen. Freshman and some upperclassmen arrived for indoctrination, then 15–18 days later most upperclassmen arrived. The major peaks of illness were centered around the end of June and beginning of August for freshman, then about 15–18 days later in the middle of August for upperclassmen. Used with permission by the author and University of Chicago Press.[12]

5.2. Age Distribution

The incidence of mycoplasma pneumonia is greatest among school-aged children and declines after adolescence.[143,144] Rates of disease among children under 5 years have been reported to be low, but these studies were conducted at a time when out-of-home child care was less common. It is likely that the rate among preschool children is higher than once thought because of the ease with which young children share respiratory secretions.

Although most mycoplasma infections occur among outpatients, *M. pneumoniae* is a significant bacterial cause of hospitalizations for pneumonia in the United States, especially among the elderly.[15] Most reports of severe complications and deaths from mycoplasma infection occur in this age group. Children may also represent an asymptomatic reservoir of infection for outbreaks in families.[145]

5.3. Transmission in Families

Mycoplasma infections are frequently spread among family members.[145–149] In a recent study in the Netherlands, 15% of family members of *M. pneumoniae*-positive index patients had organisms isolated from their nose or throat within 4 weeks of the index case.[145] The frequency of transmission within families was the same regardless of the appropriateness of the antibiotic used for treatment of the index patient. The frequency of transmission reported in this study probably represents a marked underestimate of the risk of transmission within families, given that other studies have shown that several cycles of 3–4 weeks may be necessary before intrafamily transmission is complete. In a study in Seattle, 39% of family contacts eventually became infected, many asymptomatically.[148]

6. Mechanisms and Routes of Transmission

Transmission of *M. pneumoniae* usually requires close contact, such as in a family residence or in an institutional setting with a high density of people. Transmission is believed to be by respiratory aerosols or droplets.

7. Pathogenesis and Immunity

7.1. Incubation Period

In view of the intimate contact needed for droplet transmission and the slow 6-hour generation time of *M. pneumoniae*, 1–3 weeks incubation for each case is typical (see

Figure 2). In families, several cycles of infection may be necessary before intrafamily transmission is complete. Some studies have reported incubation periods from common source outbreaks as short as 4 days,[150] whereas others have reported longer incubations with a median of 23 days with intrafamilial spread where smaller inocula may be involved and transmission may be less effective until the index case has exhibited symptoms for several days.[148]

7.2. Pathogenesis

M. pneumoniae is a mucosal parasite primarily involving the upper and lower respiratory tract, but it has been isolated and/or demonstrated by PCR in other sites including the urogenital tract, cerebrospinal fluid, synovial fluid, pericardial fluid, and cutaneous lesions.[5] In postmortem examinations, *M. pneumoniae* has been recovered from the blood in the heart and pericardial sac,[151] spleen, brain, and kidney.[79] However, the frequency of direct invasion of these sites is unknown because the organism is rarely sought for clinical purposes. *M. pneumoniae* can be isolated from the pharynx 1 week prior to onset of clinical illness; recovery rates are highest during the first week of illness, shedding from the pharynx subsequently decreases, but prolonged carriage may occur in an unknown percentage of persons. The organism has been recovered as late as 4 months after the onset of illness and for a much longer period in hypogammaglobulinemic patients.[152]

Attachment of *M. pneumoniae* to the respiratory epithelium by its specialized attachment organelle is a prerequisite for later events that culminate in production of disease.[4] The close interaction between the mycoplasma and host cells protects it from removal by the host's mucociliary clearance mechanism and allows it to produce a variety of local cytotoxic effects. The attachment organelle consists of a specialized tip structure with a central core of a dense rod-like central filament surrounded by a lucent space that is enveloped by an extension of the organism's cell membrane. The tip structure is actually a network of adhesins, interactive proteins, and adherence accessory proteins that cooperate structurally and functionally to mobilize and concentrate adhesins at the tip of the organism. Attachment organelles can be seen in a scanning EM photograph in Figure 3.

The P1 adhesin is a 170-kDa protein concentrated in the attachment tip that is now known to be the major structure responsible for interaction of *M. pneumoniae* with host cells.[5] A variety of proteins are believed to participate in the establishment of the polar structure. Once this polar structure is established, an independently assembled complex of proteins B, C, and P1 is drawn to the structure to complete formation of the functional terminal attachment organelle.[153] Attachment has been studied in detail in the hamster model and in tracheal organ culture. Ciliary movements ceased after a couple of days; the latter effect was not observed with nonpathogenic mycoplasmas. An infiltrate adjacent to the bronchial regions consisted primarily of mononuclear cells and some cells defined as plasma cells.[154]

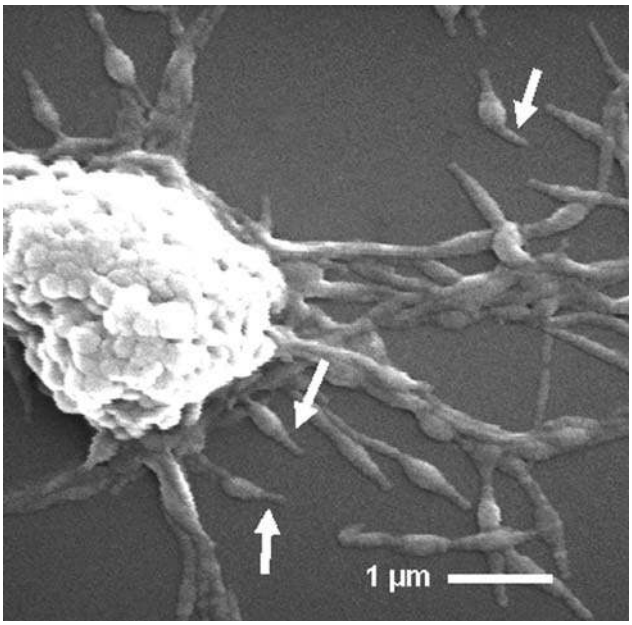


Figure 3. Scanning electron microscopic image of *M. pneumoniae* cells. A large clump of cells is present to the left. Individual cells with attachment tip organelles (*white arrows*) and typical morphology are seen (courtesy J. Carr, Centers for Disease Control and Prevention).

An important area of research is whether intracellular infections of host cells by *M. pneumoniae* and other mycoplasmas is an important factor in pathogenesis. An intracellular existence could facilitate the establishment of latent or chronic states, circumvent mycoplasmacidal immune mechanisms, facilitate the organisms' abilities to cross mucosal barriers, gain access to internal tissues, and impair efficacy of some drug therapies, accounting for difficulty in eradication.[154–157] Fusion of the mycoplasmal cell membrane with that of the host could result in release of various hydrolytic enzymes produced by the mycoplasma as well as insertion of mycoplasmal membrane components into the host cell membrane, a process that could potentially alter receptor recognition sites, affect cytokine induction and expression, and provide a mechanism for post-infectious autoimmune reactions. At present, the extent to which *M. pneumoniae*invades and replicates intracellularly in vivo is not known. Therefore, the clinical significance

of these theoretical events associated with cell fusion remains to be proven.

Close approximation of the organism to the host cells facilitated by the adhesin proteins appears to be important to facilitate localized tissue disruption and cytotoxicity. Hydrogen peroxide and superoxide radicals synthesized by mycoplasmas act in concert with endogenous toxic oxygen molecules generated by host cells to induce oxidative stress in the respiratory epithelium.[154] Hydrogen peroxide has been known to be important as a virulence factor in *M. pneumoniae* since Somerson showed it to be the molecule that confers hemolytic activity.[158] The ultrastructural effects of peroxide on host cells such as erythrocytes include loss of reduced glutathione, denaturation of hemoglobin, peroxidation of erythrocyte lipids, and eventually lysis of the cells. Superoxide anion produced by *M. pneumoniae* acts to inhibit catalase in host cells, thereby reducing the enzymatic breakdown of peroxides produced endogenously and rendering the host cell more susceptible to oxidative damage.[159]

Although the number of fatalities due to *M. pneumoniae* infection coming to autopsy is small, the postmortem findings are similar to those seen in the hamster model: interstitial infiltrates surround bronchi and bronchioles.[5] The infiltrates consist primarily of mononuclear cells, especially macrophages, lymphocytes, and plasma cells. The alveolar lining cells are swollen. Exudate, consisting of fibrin and leukocytes, has been observed primarily in the bronchial spaces.

7.3. Immunity

Elements of the host innate immune system are activated by *M. pneumoniae* and the attendant inflammation accounts for many of the initial signs and symptoms accompanying the early stages of the evolving infection. Upon reaching the lower respiratory tract, the organism may be opsonized by complement or antibodies. Macrophages become activated, begin phagocytosis, and undergo chemotactic migration to the site of infection. Therefore, it is possible that complement plays a significant role in inhibiting growth of the organism, particularly after inflammation induces infiltration by inflammatory cells and exudation.[160,161]

Adaptive immunity by both B and T lymphocytes has a major impact on the progression of mycoplasmal respiratory disease. But immunity is often short-lived, and humans are susceptible to repeated infections despite the development of complement-fixing and growth-inhibiting antibody responses. Immune responses that develop after infection often fail to eliminate the organisms, implying that adaptive immunity can have a limited effect on the clearance of an established infection, thus allowing asymptomatic carriage.[162]

Cytokine production and lymphocyte activation can reduce infection and reduce inflammation or exacerbate disease through the development of immunologic hypersensitivity, resulting in damage to the respiratory epithelium and deeper tissues. Lymphoid infiltrations undoubtedly contribute to the development of inflammatory responses. CD4+ T lymphocyte, B lymphocyte, and plasma cell lung accumulations in the lung result in radiographic manifestation of pulmonary infiltrates and are associated with production of immunoglobulins and the release of a variety of cytokines.[163]

8. Patterns of Host Response

8.1. Clinical Features

Host response to *M. pneumoniae* ranges from asymptomatic infection to pharyngitis, bronchitis, bronchiolitis, croup, tracheobronchitis, pneumonitis, and pneumonia (Table 2). The onset of mycoplasma pneumonia is usually insidious, in contrast to the abrupt onset of classic pneumococcal pneumonia. The symptoms include chills (55%), headache (66%), sore throat (54%), malaise (89%), and cough (99%). The disease may mimic infections caused by *C. pneumoniae*, various viral respiratory diseases such as adenovirus and influenza, and even the common cold. In contrast to most viral respiratory diseases, frank coryza is infrequent. Cough is characteristically non-productive and irritating at onset and may be paroxysmal. In young children, it may have a staccato character and be exaggerated at night. Wheezing is common.

A great variety of respiratory and non-respiratory complications have been described in Section 4, Etiologic Patterns.

8.2. Diagnosis

A diagnosis of mycoplasma pneumonia should be suspected in persons with gradual onset of classical clinical respiratory signs. The white blood cell count is usually normal or slightly elevated and the sedimentation rate may be high, but there are no routine laboratory tests that will provide a specific diagnosis. Dullness is rarely present on examination of the chest, since consolidation is seldom complete. Only a few rales may be heard despite an extensive infiltrate on the chest film. The radiological infiltrate is more often unilateral (84%) than bilateral. It is often described as segmental and patchy and with punctate mottling. Hilar infiltrates are common and characteristically fan out in a wedge-shaped

manner, but any configuration and location of the infiltrate is possible, including upper lobar infiltrates.[5]

Epidemiological clues may be helpful in making the diagnosis of mycoplasma pneumonia because pneumonia of other etiologies is infrequent among persons 15–29 years of age, and a high proportion of pneumonia is due to *M. pneumoniae* in this age group. Likewise, a high proportion of pneumonia encountered in the summer is due to *M. pneumoniae*, because other respiratory agents are then at a low ebb. A history of other family members or contacts having had similar disease about 3 weeks previously is also suggestive.

Isolation of *M. pneumoniae* from respiratory secretions, throat, or nasopharyngeal swabs of individuals with classic respiratory signs is diagnostic, although serologic or other confirmatory tests are useful. However, it may take 4 weeks or longer for the laboratory to grow the organism, and culture is often available only in reference laboratories. Relative advantages and disadvantages of culture, PCR, and serology for specific diagnosis of *M. pneumoniae* infections have been described in previous sections.

9. Control and Prevention

9.1. Treatment

Treatment with tetracyclines, macrolides, and ketolides shortens the duration of illness in *M. pneumoniae* pneumonia. Fluoroquinolones such as levofloxacin, gatifloxacin, and moxifloxacin are also effective.[5] Despite the patient's clinical improvement, shedding of the organisms can continue unabated. Although antibiotic treatment does not eradicate the organism, it is possible that the number of excreted organisms decreases. Patients with hemolytic anemia may require transfusion of blood, preferably I-erythrocytes (anti-I can be associated with cold agglutinin hemagglutinin disease), and the blood should be warmed to prevent hemolysis due to cold agglutinins in the host.

Because of the evidence for an immune component in some cases of *M. pneumoniae* CNS disease and because of the benefits of steroid therapy in many CNS syndromes, steroids are frequently used in combination with antibiotics to treat *M. pneumoniae* CNS infections. A 2002 review of steroid therapy in mycoplasma CNS cases suggests that it is often beneficial, particularly in severe cases. Of 14 cases of severe mycoplasma CNS disease, 11 (78%) were reported to have a complete or near-complete recovery, a better outcome than a report on an earlier series of patients who did not receive steroids.[164] Another report documents successful recoveries in patients with fulminant CNS disease that received high-dose steroid therapy early in the course of disease, leading to suggestions that steroid therapy be initiated early in the course of illness.[165] Although full recoveries are reported, several case reports document patients who relapsed with clinical signs following cessation of a first round of steroids.[83,164] This led Carpenter to suggest the use of a long taper period when steroids are used.[164]

9.2. Vaccines

Interest in development of a vaccine for *M. pneumoniae* has been evident since the early 1960s, soon after the organism was successfully isolated in culture. This interest was fostered by the lack of natural protective immunity following primary infection, prolonged carriage, and propensity for outbreaks of infections in military camps, schools, and hospitals. Development of a vaccine also seemed promising in view of the fact that the organism is rather homogeneous antigenically and there appears to be some protection against reinfection.[5,166,167] Animal models have been used extensively to improve understanding of the natural immune response to *M. pneumoniae* infection and how it might be modified by immunization.[168–179] Ellison provided an in-depth discussion of protective antigens, protective immune mechanisms, and prior vaccine strategies for *M. pneumoniae*, summarizing the work performed in this area through the early 1990s.[180]

Considering that attachment and initiation of local damage at the cellular and subcellular levels are responsible for *M. pneumoniae* disease, the logical vaccine strategy is to prevent attachment and thereby prevent initiation of disease. The initial studies in human volunteers used formalin-inactivated vaccines, but their protective efficacy results were generally disappointing for a variety of reasons that have been explored in-depth by Ellison.[180] Perhaps the most significant event related to volunteer immunizations was the experience of some immunized volunteers who developed more severe illness after experimental challenge with live mycoplasmas. This occurrence has also been documented in animal models after administration of inactivated as well as component vaccines,[180,181] signifying that immunization sensitizes the host in some way through a cell-mediated immune response. Development of live attenuated vaccines administered intranasally or by aerosol inhalation in animal models showed some protective efficacy against disease when rechallenged with virulent strains, suggesting that stimulation of local mucosal defense mechanisms is important.[160,174,180] Development of a serum antibody response after mucosal immunization may or may not occur, depending on the immunogen.[170,173,180] Live attenuated vaccines have never made it to human use due to concern

over residual virulence of the vaccine strain of *M. pneumoniae*. Similar problems were encountered with temperature-sensitive mutant vaccines.[169,180] Other vaccine candidates have included acellular protein and polysaccharide components and recombinant DNA.[182–186] The importance of the P1 adhesin in mediating *M. pneumoniae* cytadherence and initiation of disease is clear, but animal studies using P1 as a vaccine antigen have not demonstrated protective efficacy.[160,180,187,188] Experimental animal studies involving mice,[173,175] hamsters,[178] guinea pigs[189] and chimpanzees[174,178] have continued, but to our knowledge there have been no recent clinical trials in humans with any newer versions of *M. pneumoniae* vaccines and no such trial appears to be forthcoming soon.

10. Unresolved Problems

Clinical trials of new antimicrobial agents for community-acquired pneumonias such as the new macrolides and quinolones have rarely included specific procedures for mycoplasma detection, and those that did often used indirect measures of infection such as serology. Consequently, a better test of "cure" is needed to develop optimal antibiotic treatment therapies for mycoplasmal pneumonia.

The pathogenesis of extrapulmonary syndromes attributed to *M. pneumoniae* infections needs further investigation. Are such syndromes related to direct invasion or immunologic sequelae? It seems likely that either mechanism may occur.

PCR offers promise as a tool for antibiotic trials and studies of extrapulmonary syndromes as well as more rapid diagnoses, but methods need to be standardized and made commercially available for widespread use. Several products are already sold commercially in Europe, but none are available so far in the United States.

Considering not only the respiratory disease caused by *M. pneumoniae* but also the many complications, a vaccine seems desirable and should be encouraged.

11. Other Human Mycoplasmas

11.1. Background, Classification, and Disease Associations

In 1954 Shepard provided the first description of "T-strain" ("T" for tiny) mycoplasmas, later known as ureaplasmas, when he was able to cultivate them in vitro from the urethras of men with nongonococcal urethritis (NGU).[190] *M. genitalium* was first described in the early 1980s when it too was isolated from the urethras of men with NGU.[191] *M. fermentans* is an organism which may play a role in human disease in some circumstances. Evidence has accumulated in recent years to suggest that it may have an etiologic role as an opportunist in persons with HIV infection and AIDS or other immunocompromised persons (reviewed in[31]) and a possible association with chronic arthritic conditions.[192,193] Other organisms such as *M. penetrans* appear to have the potential for being human pathogens,[194] but no conclusive proof demonstrating this has been offered to date. The most recent human mycoplasmal species to be recognized is *M. amphoriforme*, an organism that has been detected in the lower respiratory tract of several immunocompromised persons in association with chronic bronchitis, and investigations are now underway to determine whether a role in human disease can be established with certainty.[195]

Ureaplasma spp. can be found on the mucosal surfaces of the cervix or vagina of 40–80% of sexually mature asymptomatic women, whereas *M. hominis* may occur in 21–53%. The incidence of each is somewhat lower in the urethra of males. Colonization is linked to younger age, lower

Table 3. Diseases Associated with or Caused by *Ureaplasma* Species, *Mycoplasma hominis,* and *Mycoplasma genitalium* in Humans

Disease	*Ureaplasma* spp.	*M. hominis*	*M. genitalium*[a]
Male urethritis	+	−	+
Prostatitis	±	−	±
Epididymitis	±	−	−
Urinary calculi	+	−	−
Pyelonephritis	±	+	−
Bacterial vaginosis	±	±	−
Cervicitis	−	−	+
Pelvic inflammatory disease	−	+	+
Infertility	±	−	±
Chorioamnionitis	+	±	−
Spontaneous abortion	+	±	−
Prematurity/low birth weight	+	−	−
Intrauterine growth retardation	±	−	−
Postpartum/postabortion fever	+	+	−
Extragenital disease (including arthritis)	+	+	+
Neonatal pneumonia and lung disease	+	±	−
Neonatal bacteremia and meningitis	+	+	−

− no association or causal role demonstrated.
+ causal role
± association and/or strong suggestive evidence, but causal role not proven in prospective studies
[a] In the case of *M. genitalium,* lack of disease association may reflect the fact that insufficient studies using appropriate detection techniques have been attempted.

socioeconomic status, sexual activity with multiple partners, African-American ethnicity, and oral contraceptive use.[196]

There is now ample evidence that *M. hominis*, *M. genitalium*, and *Ureaplasma* spp. play etiological roles in a variety of urogenital diseases of men and women as summarized in Table 3. For some conditions such as NGU for *Ureaplasma* spp. and pelvic inflammatory disease (PID) for *M. hominis*, Koch's postulates have been fulfilled and a portion of clinical cases of these entities are known to be caused by these respective organisms.[197] However, attempts to link inflammatory diseases of the upper urogenital tract with isolation of the organisms in the lower tract are not always successful, complicating our understanding of their significance as pathogens in many conditions. The discrepancy sometimes observed between the presence of genital mycoplasmas that frequently inhabit the lower urogenital tract in healthy persons and disease in the upper tract is apparently due to the fact that upper tract colonization and disease occur in only a subpopulation of persons who are colonized in the lower tract and that the reasons and risk factors for such upper tract involvement are unknown.[197]

Soon after ureaplasmas were first identified and characterized it became apparent that these organisms could be subclassified into several distinct serovars. The number of serovars was eventually expanded to 14.[198] Additional study over several years, using data obtained from 16S rRNA sequencing, led to the further breakdown of the 14 serovars into 2 biovars or clusters. Biovar 1, also known as the parvo biovar, contains serovars 1, 3, 6, and 14, while biovar 2, also known as the T960 biovar, contains serovars 2, 4, 5, 7, 8, 9, 10, 11, 12, and 13. Recently, the two biovars were designated as distinct species. Biovar 1 became *U. parvum* and biovar 2 became *U. urealyticum*.[199–204] *U. parvum* is the more common of the two biovars isolated from clinical specimens, but both species may occur simultaneously in some people.

Availability of powerful typing techniques such as the PCR assay and a shift in focus from the serovar level to the biovar or species level has led to new information concerning differential pathogenicity among ureaplasmal strains in several recent studies. There was no difference in pregnancy outcome and magnitude of intra-amniotic inflammatory response, chorioamnionitis, birthweight, or gestational age at delivery or neonatal morbidity in women whose amniotic fluid contained ureaplasmas detected by PCR attributable to biovar.[205] However, Deguchi found a significantly higher prevalence of *U. urealyticum* as compared to *U. parvum* in NGU for which prevalence was not significantly different from men without NGU.[206] They also determined it was only certain serovars of *U. urealyticum* that were associated with NGU in a subsequent study.[207] *U. urealyticum* has also been found to be dominant in patients with PID, as well as in patients who had had a miscarriage, and it seemed to have more adverse effects on pregnancy outcome, as assessed by birth weight, gestational age, and preterm delivery, than did *U. parvum* according to a study performed in Germany.[208]

Some studies have been unable to attribute a greater risk of developing bronchopulmonary dysplasia (BPD) among ureaplasma-colonized infants and also found no differences between infants harboring *U. parvum* as compared to those with *U. urealyticum*.[209] However, others have reported that 56% of infants colonized by *U. urealyticum* developed bronchopulmonary disease versus 25% of infants with *U. parvum*.[208] Katz found no significant difference or trend in prevalence of either species in infants with or without bronchopulmonary disease, but there was a significantly greater likelihood of disease among infants whose endotracheal aspirates were positive for both species.[210] Additional studies must be performed to determine conclusively the extent to which any differential pathogenicity of *Ureaplasma* species occurs in clinical conditions known to be associated with these organisms, and under what circumstances. Availability of real-time PCR assays may prove to be very useful in determining their respective roles in disease.[211]

11.2. Pathogenesis and Immunity

Mycoplasmas are primarily mucosally associated organisms residing in the respiratory or urogenital tracts of their hosts in close association with epithelial cells. In some species invasion of host cells occurs and the organisms reside intracellularly. Such intracellular localization may contribute to the chronicity of infections and their ability to evade the host cell immune response.[5,155,212–214] Localization and attachment on host cell surfaces are important in the ability of mycoplasmas to colonize and subsequently produce pathological lesions, even if cellular internalization does not occur. In addition to *M. pneumoniae*, organisms such as *M. genitalium* also have a flask-shaped morphology and terminal attachment organelles and knowledge of their cytadherence processes is increasing. Factors involved with the attachment of *M. hominis* and *Ureaplasma* spp. to mucosal surfaces have not been extensively characterized. These organisms do not have the prominent attachment tips described in the other species mentioned above, but some investigation has been done in this area. A major adhesin protein, also known as the variable adherence-associated antigen (Vaa) may undergo antigenic variation and assist *M. hominis* in evasion of host immune defenses.[215–217] The Vaa antigen is expressed in vivo during chronic active arthritis associated with *M. hominis* infection and is highly immunogenic in the human host.[218]

Similar to what has been described for the Vaa antigen in *M. hominis*, the MB antigen of *Ureaplasma* spp.

undergoes a high rate of size variation in vitro and is size variable on invasive ureaplasma isolates.[215–217] The MB antigen contains serovar-specific and cross-reactive epitopes and is a predominant antigen recognized during human infections.[219]

Ureaplasmas are known to adhere to a variety of human cells including erythrocytes,[220] spermatozoa,[221] and urethral epithelial cells.[222] Ureaplasmas bind spontaneously to neutrophils and directly activate the first component of complement.[223,224] There may be several proteins involved in the cytadherence process of ureaplasmas, which has not yet been characterized in its entirety.[220,225] The receptors for ureaplasma adhesins are believed to be sialyl residues and/or sulfated compounds, similar to what has been observed for other mycoplasmas.

Arginine metabolism by *M. hominis* and urease activity in ureaplasmas have been suggested as potential virulence factors. Release of NH_3 in the urinary tract by ureaplasmas can cause elevation of urinary pH and precipitation of magnesium ammonium phosphate, also known as struvite. Immunoglobulin (Ig) A1 protease activity was first described in *Ureaplasma* spp. more than 20 years ago.[226] Since IgA is the predominant immunoglobulin secreted at mucosal surfaces, IgA proteases may facilitate colonization by microorganisms by degrading this important component of the mucosal immune system. However, Robertson et al.[226] emphasized that IgA1 protease may not be a significant virulence factor in ureaplasmas because they were able to detect its presence in men with NGU and with strains isolated from healthy persons. Attempts to identify the gene for IgA1 protease in the genome of *U. parvum* have not been successful, and there is speculation that the ureaplasma enzyme may have diverged so far from orthologues in other bacteria that they were unrecognizable or that it may have convergently evolved no recognizable similarity to other enzymes.[227] The presence of phospholipases A and C in *Ureaplasma* spp. has been suggested to be the means by which ureaplasmas may initiate preterm labor by liberating arachidonic acid and altering prostaglandin synthesis.[228–230] However, Glass and colleagues were unable to identify phospholipase activity in the serotype 3 ureaplasma strain for which the complete genome was sequenced and gave the same possible explanations as those for the apparent lack of IgA1 protease activity.[227]

An intact humoral immune system is very important to prevent infection and dissemination by genital mycoplasmas. This is quite evident by the well-known occurrences of these organisms, ureaplasmas in particular, in septic arthritis and persistent urethritis in persons with hypogammaglobulinemia and systemic infections in immunocompromised preterm neonates.[223,231,232]

11.3. Transmission

Ureaplasma spp. and *M. hominis* can be transmitted from person to person venereally, but they can also be transmitted vertically from mother to offspring by means of at least three different routes.[233] First, there can be an ascending intrauterine infection in which the organisms gain access to the amniotic sac where they multiply and are then passed into the fetal lung. This can occur early in pregnancy, even when fetal membranes are intact and infection can persist for several weeks. Fetal acquisition of these organisms can also occur through a hematogenous route through placental infection through involvement of the umbilical vessels. Finally, acquisition of these organisms by the neonate can occur through passage of an infected maternal birth canal with resultant colonization of the skin, mucosal membranes, and respiratory tract. Ureaplasmas can be isolated from the endotracheal secretions in up to 40% of newborn infants within 30 minutes to 24 hours after birth.[234–236]

Vertical transmission of *Ureaplasma* spp. has been reported to range from 18 to 88% and isolation rates vary inversely with gestational age, according to most studies.[232] Despite the likelihood that women who are colonized with genital mycoplasmas will transmit them to their offspring, the mere presence of these organisms in surface cultures of neonates is not evidence of pathogenicity. Although the presence of *Ureaplasma* spp. has been documented for long periods in the lower respiratory tract of preterm infants,[237] surface colonization of full-term infants tends to be transient and declines beyond 3 months of age.[238] Recolonization of the lower urogenital tract may occur following puberty and when sexual activity is initiated or if there is sexual abuse.[239] However, genital mycoplasmas may occasionally be isolated from the vagina of healthy prepubescent girls.[240]

11.4. Laboratory Detection

Although culture is considered the reference standard for detection of *M. hominis* and *Ureaplasma* spp., it is expensive, requires specialized media and expertise that are not widely available outside of larger medical centers, mycoplasma research or reference laboratories. Confirmed culture results can usually be available within 2–5 days, exclusive of specimen transport time and shipment if an off-site reference laboratory is used. Ureaplasmas can be positively identified to genus level by their colonial morphology and urease production. In contrast, mycoplasmal species such as *M. hominis* that produce fried egg colonies on A8 or SP4 agar may be presumptively identified based on growth rates, hydrolysis of arginine, and body site of origin, but definitive species identification requires additional tests which have histori-

cally included growth inhibition using homologous antisera, immunoblotting with monoclonal antibodies, colony epi-immunofluorescence, and, more recently, the PCR assay.[241]

Considerable attention has been given in recent years to the application of the PCR assay in primary detection of mycoplasmal and ureaplasmal perinatal infections. PCR is essential if fastidious, slow-growing organisms such as *M. genitalium* are sought and is also valuable in differentiating ureaplasmal species and serovars. Theoretical advantages of the PCR assay for detection of genital mycoplasmas include the fact that no viable organisms are necessary, its analytical sensitivity is much greater than culture, and results can be available in 1 day.

There has been interest in development of serological assays for detection of the host immune response to genital mycoplasmas. However, their ubiquity in the urogenital tracts of adults makes interpretation of antibody titers difficult, and the mere existence of antibodies alone cannot be considered significant in most circumstances. No assays for genital mycoplasmas have been standardized and made commercially available in the United States.

11.5. Treatment

Resistance to macrolides and lincosamides is variable according to species, with *M. hominis* being resistant to erythromycin but susceptible to clindamycin. For *Ureaplasma* spp., the reverse is true. Newer macrolides, azalides, and ketolides have shown activity at least as good as that of erythromycin against ureaplasmas. Tetracycline resistance has been well documented in recent years in both *M. hominis* and *Ureaplasma* spp., mediated by the *tetM* determinant which codes for a protein that binds to ribosomes, protecting them from the actions of these drugs. The extent to which tetracycline resistance occurs in genital mycoplasmas varies geographically and according to prior antimicrobial exposure in different populations, but may approach 40–50%. Information about tetracycline resistance in *M. genitalium* does not exist, but men with *M. genitalium*-positive NGU or women with cervicitis respond better to azithromycin than they do to a tetracycline, possibly because of the lower minimum inhibitory concentrations (MICs). There are very little outcome data documented in a controlled setting with microbiological studies to demonstrate efficacy of antimicrobial treatment of *M. genitalium* infections. Such information is needed in view of the growing importance of this mycoplasma in sexually transmitted disease. Other agents such as streptogramins, aminoglycosides, and chloramphenicol may show in vitro inhibitory activity against some species.

Extragenital infections, often in immunocompromised hosts, may be caused by multi-drug-resistant mycoplasmas and ureaplasmas, making guidance of chemotherapy by in vitro susceptibility tests important in this clinical setting. Eradication of infection under these circumstances can be extremely difficult, requiring prolonged therapy, even when the organisms are susceptible to the expected agents. This difficulty highlights the facts that mollicutes are inhibited but not killed by most commonly used bacteriostatic antimicrobial agents in concentrations achievable in vivo and that a functioning immune system plays an integral part in their eradication. Fluoroquinolones such as levofloxacin, moxifloxacin, and gatifloxacin tend to have greater in vitro activity than older drugs such as ciprofloxacin, but resistant strains have been reported.[242,243]

11.6. Unresolved Problems

Better techniques of detecting, identifying, and quantifying other human mycoplasmas are needed, and continuing research will facilitate optimization of methods to accurately determine antibiotic sensitivities in the laboratory. More research into the role of intracellular infections in mycoplasma diseases is required to provide a better understanding of pathogenesis for all human mycoplasma species.

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CHAPTER 26

Healthcare-Acquired Bacterial Infections

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1. Introduction

The term "nosocomial" is derived from the combination of Greek *nosos* (disease) with *komein* (to take care of) as *nosokomeion* (hospital) and the Latin *nosocomium* (hospital). Nosocomial infections, then, are infections that develop and are recognized in patients and personnel in hospitals. These infections are not present or incubating on admission, with the exception that a nosocomial infection may be present on admission if it is directly related to or is the residual of a previous hospitalization. The reader may note that the title of this chapter has changed in a small but important way from the prior edition. We have acknowledged substantial changes in the field by revising the title from focusing on "nosocomial" infections to "healthcare-acquired" infections. As a greater number of individuals are cared for in non-acute-care health facilities (e.g., skilled nursing facilities, long-term care facilities) an understanding of infections in these settings is also vital. It has been increasingly recognized that infections which develop in such non-hospital, healthcare settings are very similar with regard to etiology and epidemiology to infections which occur in the hospital.[1] Although we will continue to use the term "nosocomial" to refer to infection occurring specifically in the hospital setting, the term "healthcare acquired" is increasingly accepted as a more inclusive term to describe infections that occur in the hospital and other healthcare settings. This reflects an identical change made several years ago by the Society for *Healthcare* Epidemiology of America (SHEA).

In hospitals alone, healthcare-acquired infections account for an estimated two million infections annually.[2] It is estimated that over 5% of all hospitalized patients fall victim to these infections each year in the United States.[3] Prevalence studies conducted in other countries with collaboration by the World Health Organization (WHO) have found rates from 3 to 20%, with a mean of 8.7 infections per 100 patients surveyed.[4] One-third to one-half of all nosocomial infections may be preventable.[5] Approximately 90% of reported healthcare-acquired infections are of bacterial etiology, with viral, fungal, protozoal, and other classes of microorganisms associated with a lesser but significant number of cases.[3] Healthcare infections exact a tremendous toll both in morbidity, mortality, and costs.[2] Hospital infections are estimated to account for 90,000 deaths and 4.5 billion dollars in excess healthcare costs annually.[2] Indeed, the number of nosocomial infections per 1,000 patient days has actually increased in the past two decades.[2] Several factors, including newer instruments and devices, more invasive procedures, sicker patient populations, stretched resources, and new patterns of antimicrobial resistance suggest that healthcare infections will continue to increase in the future.

2. Historical Background

Since the use of temples by the Egyptians, there have been documented attempts to provide special care, protection, and segregation for the sick. This gathering of the sick has frequently resulted in rapid transmission of infectious

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agents among patients and personnel, and from unrecognized reservoirs within the institution with resultant high morbidity and mortality. Public recognition of hospitals as "pesthouses" was common up to modern times. The role of improved environmental sanitation and the importance of hygienic practices in reducing transmission and infection rates were recognized in the earliest medical traditions and were periodically rediscovered in the following centuries. The medical workers of the middle 1800s were especially productive. Florence Nightingale introduced broad hospital hygiene, transmission of puerperal fever was recognized by Semmelweis and controlled by hand washing with an antiseptic, and Lister introduced antisepsis to surgery. After Pasteur, the putrefaction associated with trauma and surgery was understood as related to transmissible microorganisms, and the importance of aseptic technique became well understood and practiced.[6,7]

The stunning early success of penicillin in controlling gram-positive coccal infections led to the misconception that antibiotics could control and eventually eradicate all infectious diseases. The carefully learned lessons of the past concerning aseptic technique were no longer given priority and support. The appearance of penicillin-resistant staphylococci in this milieu led to devastating hospital epidemics with these agents.[8] With the discovery of penicillinase-resistant penicillins, infections by resistant staphylococci became treatable, and associated epidemics decreased in importance. At the same time, the marketing of antibiotics with effectiveness against a broad spectrum of other microorganisms again led the unwary to discount the need for other control methods. However, the control of healthcare infections was not forthcoming. Indeed, recent years have witnessed the emergence and global spread of a wide variety of antimicrobial resistant pathogens such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, and multidrug-resistant *Pseudomonas aeruginosa.*[9] These trends are all the more concerning given the association between antimicrobial resistant infection and negative clinical and economic outcomes,[10] as well as the recognized slowing of development of new antibacterials over the past decade.[11]

3. Methodology

3.1. Criteria for Infection

Widely accepted criteria for the identification and classification of nosocomial infection have been suggested by the Centers for Disease Control and Prevention (CDC).[12,13] These criteria satisfy both the need for scientific validity and the practical requirement for easy and uniform recognition necessary to discovery and enumeration. Examples are listed in Table 1. It is common practice to designate most bacterial infections that appear within the first 48 hour of hospital stay as community and not nosocomially acquired. In these criteria both infections from endogenous flora and infections transmitted from exogenous sources are included. Since microbiological sampling in clinical situations is neither routine nor uniform, the criteria are not dependent on the laboratory for data for fulfillment. Use of a clinical definition is mandated by studies indicating that from 16 to 59% of otherwise well-documented nosocomial infections are not supported by microbiological data in that no tests were submitted or tests were invalidated because of poor sampling, inadequate handling, or laboratory error.[4,14,15] Since both preventable and unavoidable infections are included in these criteria, care must be taken in assignment of causation and liability in the individual case.

3.2. Sources of Mortality Data

The usual hospital discharge and death certificate data rarely include comments on complications of nosocomial infection. Some individual studies[16,17] and an autopsy

Table 1. Sample Criteria for Determination of Nosocomial Infection[a]

Site	Clinical data	Laboratory data
Urinary tract	Suprapubic tenderness, dysuria, frequency, pyuria	Standard quantitative clean-catch urine culture[b]
Wound	Purulent drainage	Wound culture[b]
Respiratory tract	Physical signs, sputum change	X ray
	Purulent sputum	Sputum culture[b]
Intravenous	Purulent drainage, pain, erythema	Semiquantitative culture[b]
Gastrointestinal tract	Diarrhea	Stool culture[b]
		Toxin assay
Skin	Purulent drainage, physical signs	Culture[b]

[a]Modified from Garner et al.[12]
[b]Combinations of clinical data may be used to document infection in thr absence of laboratory microbiological isolation (see the text).

study[18] have been reported. Autopsy data may provide confirmation or documentation of unsuspected nosocomial infection through gross, microscopic, or microbiological cultural evidence. However, autopsy data are biased for patients dying in the hospital with the most severe or puzzling illnesses. These factors are associated with prolongation of hospital stay and other comorbid events predisposing to increased nosocomial infection. Since 1970, the National Healthcare Safety Network (NHSN) of the CDC (formerly known as the National Nosocomial Infections Surveillance [NNIS] system) has reported selected mortality and morbidity data from a volunteer group of hospitals in the United States, currently numbering approximately 300 hospitals.[3,9,19–22] These hospitals range in size from 100 beds to over 1,000 beds but are not geographically representative of all US hospitals. Furthermore, hospitals in the NHSN are more likely to represent larger, academic medical centers. While the system does not represent a probability sample of US hospitals, by stratifying hospitals by size and teaching characteristics, it has provided useful data. The NHSN data have tended to confirm the findings of the Study of Efficacy of Nosocomial Infection Control (SENIC) and other published reports.[23,24]

3.3. Sources of Morbidity Data

The uniform hospital discharge summary rarely indicates the complication of a nosocomial infection. In a study of risk factors important in nosocomial infection, Freeman and McGowan[25] found only 59% of nosocomial infections labeled with any diagnosis of infection as a primary or secondary discharge diagnosis and only 2% labeled as clearly indicating infection of a nosocomial origin. In a study conducted after the beginning of the new prospective payment systems in the United States, Massanari et al.[26] found that only 57% of nosocomial infections were properly recorded in the discharge abstracts of their university hospital. The retrospective audit of medical records to determine the frequency of nosocomial infection has been complicated by difficulties in completeness, illegibility, and inaccessibility. However, the results of a large, statistically complex, and well-controlled evaluation of the efficacy of nosocomial infection practice and control, using a measured and highly reliable retrospective medical record review methodology, have been reported by the CDC. The methodology and analysis of the SENIC project have been published in detail.[27,28]

The focal point of hospital infection control must be the patient. The most successful acquisition of data on nosocomial infection has been accomplished through nosocomial infection surveillance. Of critical importance is a specifically trained individual, the infection control practitioner or nurse epidemiologist.[29,30] This surveillance method actively acquires data from multiple laboratory[31,32] and clinical sources and uses it to fulfill criteria that define the presence of a nosocomial infection at a particular site. These traditional data sources have included concurrent review of the patient's clinical record, review of the medical nursing Kardex and other data sheets for evidence of infection, and coordinated supplement of these clinical sources with radiology reports, pharmacy data, and microbiological laboratory reports.[29,31–34] Advances in information technology have increasingly allowed a more systematic and streamlined approach to establishing baseline rates of infection and setting triggers for evaluation of a possible outbreak.[35,36]

Historically, nosocomial bacterial infections have not been reportable through any routine public health system. However, after years of discussion on both the federal and the state level, mandatory reporting of healthcare-associated infection rates has become a reality. Currently, mandatory reporting is required by four states, and legislation is pending in over 20 additional states.[37] Consumer demand for greater information regarding issues such as healthcare-associated infections has increased since the 1999 publication of the Institute of Medicine's "To Err is Human: Building a Safer Health System," which reported 98,000 deaths and $20 billion dollars spent per year in US hospitals associated with medical error.[38] Entities such as the Centers for Medicare and Medicaid Services (CMS), consumer groups, quality improvement and patient safety organizations, state and federal legislators, and the National Quality Forum are among those pursuing this issue on various levels. Professional organizations, including the Association For Professionals In Infection Control and Epidemiology (APIC), The Healthcare Infection Control Practices Advisory Committee (HICPAC) (a federal advisory committee that provides advice and guidance to the Department of Health and Human Services and CDC), and the Society of Healthcare Epidemiology of America (SHEA) have all issued statements and published reports relative to the issue of mandated public reporting.[39,40] To this point, the specific healthcare-associated outcomes to be reported are still being debated. The impact of public reporting has not to date been rigorously evaluated.

Another new trend in surveillance programs is "syndromic surveillance" which is used to track potential agents of bioterrorism and newly emerging diseases (e.g., severe acute respiratory syndrome).[41] These data would be routinely transmitted to the public health authorities as an "early warning" system for a bioterrorism event. The efficacy of such reporting systems has not been rigorously evaluated. Furthermore, the requisite components of such a reporting program remain unclear.

3.4. Surveillance

In addition to the NHSN and SENIC projects of the CDC, some statewide incidence and prevalence studies have been reported in the United States,[42] and the WHO and others have sponsored surveys in other regions of the world.[4,43–45]

3.4.1. Microbiological Surveys. Data routinely generated in the hospital microbiology laboratory have been widely used as a source of information of infections in hospitalized patients, and the increased use of diagnostic tests has been documented to increase the recognition of infectious diseases in acute-care institutions[23,46] The site of infection (e.g., urinary tract, blood, pulmonary, wound), patient location, causative organism(s), and antimicrobial susceptibility data can be determined and evaluated. Computerized data-processing methods have eased the collation and review of these data for recognition of clusters of infections at specific sites, in geographic locations, or of specific species or strains of bacteria.[31,32,47]

One of the most important activities performed by an institution's clinical microbiology laboratory is the reporting of cumulative and ongoing summaries of institutional patterns of antimicrobial susceptibilities, or antibiograms.[48–50] In addition to its critical role in identifying and monitoring patterns of emerging antimicrobial resistance,[35,51] the antibiogram is vital for making informed decisions regarding prescribing of empiric antimicrobial therapy.[52,53] The importance of optimizing empiric antimicrobial therapy has been highlighted by recent studies reconfirming a strong association between mortality and inadequate empiric antibiotic therapy.[54–56]

In 2002, the National Committee for Clinical Laboratory Standards (NCCLS) (now the Clinical and Laboratory Standards Institute, or CLSI) published guidelines to assist the preparation of antibiograms.[52] A study[57] conducted before these NCCLS guidelines became widely available noted that only 60% of surveyed US hospitals followed three of the basic guidelines previously proposed (i.e., compilation of an antibiogram, annual updating, and distribution to infection control staff and medical staff yearly).[50] More recently, a study of 65 academic institutions noted that 22% of centers update antibiograms at least every 6 months, while 78% update annually.[58] Although only 37% of centers report trends in susceptibilities over time, this practice was more common among teaching hospitals and in laboratories with a greater number of personnel.[58] Also, 63% of centers reported both inpatient and outpatient susceptibility data and 20% reported a separate antibiogram for different anatomic sites of culture.

In addition to informing empiric antimicrobial therapy, unit-specific susceptibility data may also permit more focused and prompt identification of changes in resistance to allow earlier intervention.[59,60] While fewer than 50% of laboratories report unit-specific antibiograms, this practice was significantly more common among teaching hospitals and laboratories with a greater number of personnel.[58] A recent study incorporated an assessment of unit-specific susceptibility rates into an intervention to improve empiric antimicrobial prescribing.[61]

Although 88% of centers exclude duplicate isolates when calculating susceptibility data, the time frame during which isolates are excluded was extremely variable.[58] Although it is well recognized that using different time cutoffs (e.g., 3, 7, 365 days) may result in important differences in reported susceptibility rates,[62–64] there has been little historical consensus on which criteria should be used. This lack of consistency makes interpretation of differences in susceptibility patterns across institutions more complex. The CLSI recommendations to include only the first isolate of a species per patient per analysis period (typically 1 year)[52] have been supported by recent studies as the simplest and most unambiguous approach.[65,66]

Although laboratory reports are a good source for initial case finding, the specificity of the survey of laboratory isolations is low. Isolation of an organism does not separate community acquired from nosocomial infection and does not specify causation in differentiating commensal from infecting bacteria without additional clinical information. Furthermore, the sensitivity of this system is dependent on the uniformity of the collection of specimens and of culturing practices, features of laboratory practice that are known to vary widely. However, when laboratory data are combined with information from other databases in a highly computerized hospital information system, sensitivities for the identification of nosocomial infection were reported that were equal to or exceeded other methods and at an efficiency that required approximately one-third of personnel devoted to routine surveillance.[35]

Routine microbiological surveys of hospitalized patients or the hospital environment are unnecessary and economically unjustifiable. In the absence of epidemic or outbreak situations, there is no evidence that this type of routine sampling has contributed significantly to detection or prevention of nosocomial infections. In epidemic situations, environmental cultures should be considered only if epidemiological evidence indicates an environmental source for the organism. The incidence of nosocomial infection has not been related to levels of microbial contamination of air, surfaces, or fomites, and meaningful standards for permissible levels of such contamination do not exist.[67–70]

The spread of nosocomial organisms in endemic and most epidemic situations is associated with contact transmission via personnel hands or due to contamination of

an item that should be sterile. Therefore, the role of environmental microbiological sampling is currently reduced to (1) assisting in epidemic investigation in selected circumstances, (2) evaluation of new techniques, (3) serving as an important adjunct to educational programs in the support of nosocomial infection control, and (4) quality control routine monitoring of sterilization, dialysis fluids, infant formula prepared in the hospital, and samples of certain disinfected equipment.

3.4.2. Prevalence Surveys. A prevalence survey of healthcare-associated infections based on application of uniform criteria to all patients in an institution during a single day or time period has provided a rapid, inexpensive, and corroborative means of assessing nosocomial infection risk within an institution. Periodic prevalence data have been used as an evaluative tool in infection control programs,[25] as an estimator of risk across a wide variety of institutions,[14,71] including programs of the WHO,[4] and as an important quality control feature of other data collection methods.[30] As with all prevalence studies, bias inherent in sampling of a single day's experience as an indicator of typical risk must be recognized. In particulate, because new and existing infections are counted, the rates obtained from prevalence surveys are usually higher than incidence rates.

3.4.3. Surveillance Studies. Currently the most widely propagated surveillance method for the collection of nosocomial infection data employs a trained individual, a nurse epidemiologist, or infection control practitioner.[34,72,73] This individual concurrently provides a coordinated review of each patient's clinical and laboratory data, recording those instances that satisfy the standard criteria for identification of a nosocomial infection. These data are summarized on a periodic (monthly, quarterly, yearly) basis, collated, analyzed, and reported to a specific multidisciplinary committee within the hospital and to certain key hospital personnel.[29,30] The importance of the unbiased collection of these data by a trained professional has been repeatedly documented by studies indicating reduced efficiency and accuracy of reporting by other methods[74,75] and by the results of the SENIC project.[28]

The institutional infection control committee recommends appropriate control measures, based on the priorities of risk indicated by the data previously collected and evaluated.[76–78] The infection control committee plays a vital role in ensuring patient safety through the prevention and control of infections in a healthcare facility. This committee is a mechanism for the infection control program to report activities including statistics, outbreaks, and proactive control measures. The infection control committee also drafts and disseminates policies and procedures on infection surveillance, prevention, control, and education. Infection control committees vary in that some serve single healthcare facilities (hospitals or long-term care facilities), while others are regional committees.[76–78]

The continuous collection of nosocomial infection data allows concurrent evaluation of these control efforts. This form of nosocomial infection data collection, evaluation, and recommendation has been termed "a nosocomial infection surveillance and control program."[79] This format has been widely promoted and supported by the Hospital Infections Program of the CDC since the first reports of its successful use in 1969.[30]

Three demonstration projects by the CDC have investigated this format as the prime method to investigate and control nosocomial infections in the US hospitals. The Comprehensive Hospital Infection Project (CHIP) collected intensive surveillance data in nine community hospitals between January 1970 and June 1973, using definitions and methods suggested by the CDC.[30,79] At 4-month intervals, CDC medical epidemiologists conducted onsite prevalence surveys to evaluate surveillance efficiency.

The NHSN (formerly NNIS) system, while not statistically representative of all US hospitals, does contain community, community teaching, state, local, and university hospitals.[9] Large hospitals with more than 500 beds are overrepresented and small hospitals with fewer than 200 beds are under-represented in the system.[22] Each hospital is required to have an active infection control program, but until recently there was little attempt to evaluate or control the quality of the submitted data. Recently the requirements for membership in the system have been increased, criteria for infection have been improved and standardized, and reliable electronic submission of data has been required in an attempt to improve both the scope and the quality of the information submitted. Data reported by the NHSN system are reviewed in Section 5.

In 1974, the CDC initiated the SENIC project, which thereafter collected data in 338 randomly selected US hospitals. The objectives of this study were to estimate the magnitude of the nosocomial infection problem in US hospitals, to describe the extent of hospitals' infection surveillance and control programs, and to evaluate the efficacy and effectiveness of these programs in reducing nosocomial infection risks. The extensive and complex methodology of the study was reported in 1980, and the results and evaluation were reported in 1985.[27,28,44] The results indicate that in the one-third of hospitals supporting the lowest levels of surveillance and control programs, nosocomial infections had increased in the period between 1970 and 1975–1976 by 18%. In the same time period in the 0.5% of hospitals with the highest levels of surveillance and control, nosocomial infections had decreased by approximately one-third.

Of the approximately one-third of infections estimated to be reduced by the most intensive surveillance and control

programs, surgical wound infections were found to be the risk most amenable to control and the problem that demonstrated the greatest risk of both increased cost and length of stay. Bacteremias and urinary tract infections were intermediate in reduction by CDC methods; pneumonias proved to be the most refractory, and nosocomial medical pneumonias were not being significantly reduced.

The most effective programs conducted high-level balanced surveillance and control activities, had a trained hospital epidemiologist, supported an infection control practitioner for each 250 beds, and had a system of reporting wound infection rates to individual surgeons. Unfortunately, it was estimated that only 6% of nosocomial infections were being prevented at the end of the study. A follow-up study in 1983 indicated that this figure had risen to 9%, or approximately 28% of the potential estimated to be possible by current methods.[80] Some question the applicability of the findings (particularly the adequacy of one infection control practitioner for 250 beds) to hospitals today since the amount and complexity of the work of the infection control practitioner has increased, the care of patients in the healthcare setting has become more complex, and the severity of illness of the hospitalized patient population has continued to increase.[78]

3.5. Laboratory Diagnosis

Over the past several decades, the clinical microbiology laboratory has assumed an ever increasing critical role for ongoing management and control of healthcare-associated infectious diseases. Surveillance from the laboratory can measure institution-wide occurrences from a single, central data collection point. Although the optimal approach for managing healthcare-associated infections is to gather the broadest surveillance data, a primary obstacle to achieving this goal has been that manually attempting universal surveillance is very resource intensive and the amount of data collected is overwhelming, even with any traditional computer analysis.[81] It has been estimated that 20th century approaches to analyzing laboratory data will detect not more than two-thirds of nosocomial infections.[82] However, many now believe that the use of laboratory data will prove the most cost-effective, comprehensive surveillance approach once sufficient analytical tools are available.[83]

A 1998 consensus report set the current standard for required microbiology laboratory services as part of a comprehensive infection control program.[78] The final report indicated that the necessary contribution from the laboratory included surveillance (e.g., providing for a systematic observance and measurement of disease) as well as molecular typing of microbial pathogens.[78] Present and future needs for laboratory-based surveillance will require reliable detection of new pathogens that emerge as causes of important healthcare-associated infections, which implies accurate identification of microbial organisms; recognition of new or emerging antimicrobial agent resistance; and participation in active surveillance for outbreaks (i.e., prospective assessment of colonization with organisms of interest), including preparation of specialized growth media as well as molecular typing.[84] This evolving role dictates the need for a strong collaboration between the hospital epidemiologist and the clinical microbiologist.[85]

3.5.1. Isolation and Identification of Organisms. Standard, reliable laboratory identification of bacteria in clinical samples from suspected nosocomial infection is important epidemiologically to link sources of transmitted microorganisms. The most important steps in assuring the validity of this process begin at the bedside with the collection of body fluids for testing. Inappropriate collection of specimens magnifies the usual difficulties in differentiation of normal flora of the body from truly infecting microbes. For example, pulmonary secretions collected through the oropharynx may be heavily contaminated by commensal oropharyngeal bacteria.[86] Similarly, urine specimens may become contaminated when not obtained by "clean-catch, midstream voiding" or by direct bladder catheterization. Cultures from other normally sterile sites must be obtained only after proper preparation of the location to be sampled for results of cultures to be meaningful.

A delay in transport to the laboratory or transport under inappropriate environmental conditions or in a hostile medium may hinder identification of important infecting bacteria. Urine cultures held at room temperature may allow bacteria therein to multiply beyond the usual criteria for identification of a nosocomial urinary tract infection. A collection of anaerobic organisms in cold medium and carriage at ambient oxygen levels may inhibit subsequent growth to an extent that thwarts laboratory rescue and identification.

Many laboratory findings suggest improper collection or transport of specimens. Isolation of skin flora, such as diphtheroids or coagulase-negative staphylococci, from normally sterile sites suggests contamination at the time of collection. The recovery of three or more species of bacteria from urine specimens suggests unsatisfactory technique in collection or handling. The inability to recover anaerobes seen on Gram's stain suggests inappropriate transport of specimens or culturing technique. Negative cultures for *Mycobacterium tuberculosis* in patients at high risk may suggest inappropriate sputum collection. Delayed receipt of sputum specimens diminishes the laboratory's ability to isolate pneumococci from specimens due to overgrowth of the organism by normal oropharyngeal flora. If such

inappropriate sampling, mishandling, and delay are not recognized by the laboratory, they may result in the unintentional misreporting of no organisms or of microbes of no clinical significance.

3.5.2. Evaluation of Specimens: Initial Screening. The laboratory specialist is guided by the description of the site of sampling and other clinical information forwarded by the requesting physician. The absence of such information accompanying the specimen may lead to serious mishandling or delay in identification in the laboratory. Recording the time of arrival of the specimen in the laboratory as compared to time of collection may be used to determine delays in transport that will result in an inability to culture fastidious organisms. Specimens received in improper collection containers can also be noted at the time of initial evaluation. In these instances, repeat cultures should be requested.

Assessment of specimens at the time they are received in the laboratory by direct microscopic examination is important in evaluating the acceptability of a specimen for further diagnostic testing. The laboratory use of differential cellular characteristics of sputum and saliva has been helpful in clarifying the source of specimens and their acceptability for diagnostic testing.[87] Specimens containing more than ten squamous epithelial cells per low-power ($\times 10$) field suggest contamination with oropharyngeal flora. Material from wounds that do not show polymorphonuclear leukocytes is also unlikely to yield a causative organism when cultured. Smears and other recently developed rapid diagnostic tests that evaluate antigen or antibody detection are of enormous importance in patient management. Smears are also important for detecting organisms of epidemiological significance, such as *Haemophilus, Legionella*, or *M. tuberculosis*, that may not be reflected by culture or that may take long periods of time to grow.

Sufficient sophistication in the laboratory is necessary to assure identification to the species level of the common bacteria endemic to the institution. Standard methods are available in many manuals and texts.[88] Reference bacteriology should be conveniently available to assist in the identification of more unusual microbes. The degree to which organisms are identified to the species level can also have epidemiological importance. For example, a report of *Pseudomonas* species fails to distinguish between a variety of related organisms, such as *P. aeruginosa* and *P. cepacia*, which have different reservoirs and modes of transmission. Identifying organisms as *P. cepacia* has etiologic significance, since *P. cepacia* are associated with outbreaks or pseudoepidemics caused by contaminated water or solutions.[89]

3.5.3. In Vitro Susceptibility Testing. Testing of the sensitivity of isolates to a spectrum of antibiotics is a commonly used clinical and epidemiological tool. Standardized Kirby–Bauer disk diffusion testing or the more exacting microdilution minimum inhibitory concentration (MIC) tests provide useful information regarding the susceptibility of the bacterial isolate.[88] Disk diffusion testing is performed by measuring the zone of inhibition around an antibiotic-impregnated disk. Zone diameter are interpreted in signifying susceptible, intermediate, or resistant organisms. Microdilution testing provides a quantitative measurement of the MIC, which is defined as the lowest concentration of antibiotic that results in inhibition of growth of the organism.

The results of susceptibility studies provide guidance both to the clinician for appropriate therapy and to the epidemiologist as an inexpensive screen for possible common identity of speciated organisms.[46] However, antibiotic sensitivity does not ensure similarity of speciated organisms; organisms may have similar antibiotic resistances and yet be epidemiologically unrelated.[90–92]

3.5.4. Quality Control in the Laboratory. Quality control is essential to effective microbiology laboratory and infection control programs. Pseudoepidemics due to contamination of specimens in the laboratory by automated laboratory equipment or other routes have occurred.[93,94] New procedures in the laboratory related to susceptibility testing, new culture techniques, or new ways of reporting results or of naming organisms can also lead to confusion, pseudoepidemics, or lack of recognition of related organisms.

3.5.5. Special Studies for Examining the Relatedness of Isolates. Molecular typing methods have become increasingly powerful tools for epidemiologic investigations of nosocomial infections[95] (see Chapter 3). When used as adjuncts to classical epidemiologic studies, these methods can greatly facilitate the investigation of nosocomial infections and outbreaks. A major caveat is that results of molecular typing should never be interpreted in the absence of data from sound clinical epidemiologic studies. Although molecular studies can help to prove or disprove epidemiologic hypothesis, when used inappropriately, incorrect conclusions may be drawn.

The goal of strain typing in the investigation of outbreaks is to provide laboratory evidence that epidemiologically related isolates are also genetically related and therefore are the same strain, presumably transmitted by a common source of exposure or from patient to patient.[96] Molecular techniques have been increasingly employed to detect slow-growing or fastidious organisms such as *Bartonella henselae* and *Mycobacterium tuberculosis*. A more recent use has been the rapid detection of organisms carrying antibiotic resistance genes such as those that encode for methicillin and vancomycin resistance. These techniques offer advantages in infection control and more timely appropriate treatment for patients.[97]

3.5.5.1. Plasmid Restriction Enzyme Analysis (REA). Molecular methods that analyze bacterial plasmid DNA increasingly have been used to determine relatedness of isolates. Diverse species of gram-negative and gram-positive bacteria have been studied using this approach.[91,98,99] Bacterial plasmids are extrachromosomal DNA elements present in most bacteria. Plasmids are extracted from bacteria by cell lysis and separated by agarose gel electrophoresis. In restriction enzyme analysis (REA), restriction endonucleases, enzymes that digest (cleave) DNA at particular sequences, are used to cleave the plasmids prior to separating them on a gel and comparing the numbers and sizes of the fragments. Plasmid REA is the simplest of the DNA-based methods and relatively inexpensive. It is limited by the fact that plasmids can be spread to multiple species ("plasmid outbreak"), lost spontaneously, or altered by mobile genetic elements that they frequently carry.

3.5.5.2. Chromosomal Restriction Enzyme Analysis (REA). The chromosome is a stable marker of strain identity. This method allows a larger amount of genetic material to be compared than with plasmid typing. The technique has been particularly useful for bacteria that lacks plasmids. The extracted chromosomal DNA is cleaved with a restriction enzyme, and the resulting linear fragments of DNA are separated by agarose gel electrophoresis. Different strains of the same bacterial species have different REA profiles because of variations in DNA sequences that alter the frequency and distribution of restriction sites. The advantage of this technique is that all isolates are typeable by REA. The disadvantage, however, is the difficulty in interpreting complex profiles that may contain hundreds of unresolved and overlapping bands. REA patterns may also be confounded by the presence of contaminating plasmids.

3.5.5.3. DNA–DNA Hybridization. The complex banding patterns generated with chromosomal DNA restriction enzyme analysis are more easily interpreted when a few discrete DNA fragments are highlighted by hybridizing the chromosomal DNA with a DNA probe that recognizes a specific gene or region of a gene that is present on a few fragments. Several bacteria have been typed with this technique, including *M. tuberculosis*. Ribotyping is another chromosomal typing technique that uses ribosomal RNA as the probe. This technique is based on the finding that the genes that encode subunits of ribosomal RNA are conserved between species and scattered throughout the chromosome of most bacteria.

3.5.5.4. Pulsed-Field Gel Electrophoresis. This technique has proven to be an excellent typing method that has been successfully used for a wide variety of bacterial species. Although special equipment is required to perform this technique, it is becoming the method of choice for typing many bacteria.[100] Guidelines with criteria for strain typing with PFGE have been published.[96] Pulsed-field gel electrophoresis allows for the separation of large genomic DNA molecules generated by rare cutting restriction enzymes. In contrast to REA, the restriction enzymes used in PFGE have fewer recognition sites in the bacterial chromosomal DNA, yielding a smaller number (10–30) of restriction fragments. The large DNA fragments are separated on agarose gels that are subjected to alternating electric fields. Relatively clear patterns that can be compared are generated in this manner.

3.5.5.5. Polymerase Chain Reaction (PCR). This powerful technique has been adapted for use as a typing tool for epidemiologic investigations. With it, very small amounts or sequences of DNA can be detected by amplifying specific nucleotide sequences and increasing the number of DNA copies. This technique permits the characterization of a "molecular fingerprint" for each organism based on its unique sequences of DNA (or RNA). The technique is much more sensitive than DNA–DNA hybridization with a specific DNA probe. Compared to other methods, PCR offers the advantage of speed (minutes to hours) and is unrivaled in sensitivity.[101] The high sensitivity of PCR can lead to its greatest limitation, in that false-positive results can occur due to amplification of contaminating DNA that may be present in a sample. Therefore, PCR should be performed only by personnel trained for the technique in a laboratory specifically set up for the procedure, and negative controls must always be used.

4. Biological Characteristics of Nosocomial Organisms

The relative frequency of the species of bacteria causing healthcare-acquired infections has changed remarkably over the past 30 years.[3,9,21,102] Changes in sensitivity to antimicrobial agents have also been observed.[9] These changes in the development and persistence of resistant strains have likely been related to the selective pressure of the widespread use and misuse of antimicrobial agents.[103] These patterns appear to be an extremely important phenomenon in local institutions and geographical areas,[104] as well as on a national level.[49,105] The biological characteristics important in these resistance changes may be generalized to (1) the ability of bacteria to survive in the normal flora of the host, (2) the ability of bacteria to survive in the animate and inanimate environments of the healthcare facility, and (3) the ability of the bacteria to survive antimicrobial stress through acquired resistance.

4.1. Infecting Normal Flora

Bacteria that normally reside as commensals on the internal and external surfaces of the host have been increasingly recognized as causal in nosocomial infections.[106–112] In hospitalized patients, the normal pharyngeal and intestinal flora may be displaced by antibiotic-resistant strains. The changes brought about by modification in the host by disease and by treatment factors occur within a few days of hospitalization. These bacteria then can serve as sources for infection in the colonized patient or are transmitted between patients nosocomially.[113] The ability of bacteria to infect is determined by the product of their number and virulence and inversely related to the quality and quantity of host defenses. In this context the term *normal flora* has become suspect in the recognition of the fact that virtually any microorganism that appears in the host may be invasive and disease causing, given a sufficiently impaired patient.[113,114]

4.2. Antimicrobial Resistance

The emergence of antimicrobial resistance has threatened to render the existing antibiotic arsenal useless. Both the number of organisms exhibiting resistance and the mechanisms of resistance have increased sharply in recent years.[115] Infections due to antibiotic-resistant bacteria are associated with increased morbidity, mortality, and hospital costs.[116,117] Of note, expenditures due to antimicrobial resistance in the United States in 1995 were estimated to be $4 billion.[118] Increasing antimicrobial resistance is of great concern not only because the agents available to treat these resistant infections are limited but also because the treatment of infectious diseases relies heavily on empiric therapy. Empiricism has become increasingly difficult as the susceptibility patterns of common pathogens become more complicated and unpredictable. Furthermore, development of new antimicrobials has slowed significantly over the past 10 years.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Many studies from the United States and other countries have estimated the magnitude of nosocomial infection by a variety of methods. Examples of data from those that have used criteria for infection compatible with those developed and used by the CDC are presented in Table 2. Based on these criteria, these studies routinely include in their totals infections for which no cultures were done or for which no pathogens were isolated. The infections identified by clinical criteria alone make up from 16 to 59% of the data.[4,9,14,27]

Historically, the nosocomial infection "rate," more precisely a ratio, has commonly been calculated as a cumulative incidence, expressed as the number of nosocomial infections per 100, 1,000, or 10,000 *patient discharges*[30,79] A preferred ratio expressed in infection per 1,000 or 10,000 *patient days of risk*[25,119–122] has been used in reports of incidence data. This incidence rate (or incidence density) is defined as the number of new infections in a specified quantity of person–time of observation among individuals at risk. This latter rate is especially valuable in comparing nosocomial risk in institutions or services with widely varying length of stay. Although this ratio accounts for length of stay, it does not take into account the use of invasive devices, which are a major risk factor for specific nosocomial infections, e.g., intravascular catheters for bloodstream infections, urinary catheters for urinary tract infections, and ventilators for pneumonias.[123] Risk-factor-specific infection rates have been developed to account for exposure to these extrinsic and powerful risk factors. The rate is expressed as the number of device-associated infections for a site per 1,000 device days of risk. These rates are considered more appropriate for interhospital as well as intrahospital comparison than the overall nosocomial infection rate, as they partially control for length of stay and device use.

5.1.1. Mortality Data. Estimates of the role of nosocomial infection in mortality have varied widely. CDC–NNIS data estimated that 0.7% of nosocomial infections directly caused death and 3.1% of infections contributed to death.[3] In a retrospective study of 1,000 autopsies from a university hospital, in which 75% of patients who died were examined, Daschner et al.[18] found an overall nosocomial infection rate of 13.7%. In those individuals with nosocomial infection, 54% of the deaths resulted directly from infection. In a retrospective study of 100 consecutive deaths at each of two hospitals, Gross et al.[17] found death causally related to nosocomial infections in 2.5% and contributing in 3.4% of patients without underlying terminal illness. In those with terminal illness on admission, the rates increase to 9.2 and 10.9%, respectively. In hospitals alone, healthcare-associated infections account for an estimated 2 million infections, 90,000 deaths, and $4.5 billion in excess healthcare costs annually.[2] Given that the severity of illness of the hospitalized patient population has continued to increase,[78] it is expected that the impact of healthcare-associated infections will continue to increase in the future.

5.1.2. Prevalence Studies. Prevalence studies conducted in US acute-care hospitals, using CDC–NNIS-compatible criteria, have reported rates ranging from 7.2% in small, predominantly rural hospitals[14] to as high as 15.5%

Table 2. Prevalence and Incidence Rates of Nosocomial Infection[a]

Location	Years	Type of hospitals	Type of study	Rate (%)
United States				
Virginia	1972–1975[24]	University	Incidence	6.7
Utah	1972–1973[14]	Community <75 beds	Prevalence	7.2
	1977[71]	Community <75 beds	Incidence	2.0
West Germany	1976[18]	University	Incidence	6.6
United Kingdom	1980[334]	Community	Autopsy	13.7
			Prevalence	9.2
Israel	1975–1976[335]	University	Incidence	12.2
United States	1984[3]	Mixed NNIS-CDC[b]	Incidence	3.4
		Community NNIS-CDC[b]	Incidence	2.2
		University NNIS-CDC[b]	Incidence	4.1
WHO	1983–1985[4]	Mixed	Prevalence	9.9

[a]Infections per 100 hospital discharges.
[b]NNIS, National Nosocomial Infections Study.

in a large, metropolitan teaching hospital[102] (Table 2). A WHO cooperative hospital infection prevalence survey was carried out between 1983 and 1985 in 55 hospitals in 14 countries in the four regions of Europe, Mediterranean, Southeast Asia, and western Pacific.[4] Results reported from 47 hospitals and 28,861 patients showed a mean prevalence rate of 8.7% with regional means from 7.7 to 11.8% and individual institutional rates from 3.0 to 20.7%. Since multiple infections were not recorded, an adjusted mean of 9.9% for the total was estimated, suggesting that approximately one patient in ten in these hospitals was infected while under care.

Prevalence data reported for infections from extended-care facilities have ranged from 2 to 18%.[124–127] These studies have used varying criteria and methodology, making comparisons difficult. Studies in these institutions require special criteria and methods different from those commonly used in acute-care institutions, since the availability of laboratory data, the extent of daily medical record documentation, and the resources available for data collection and analysis are less in this setting. Standard definitions for healthcare-acquired infections for use in extended-care facilities have been proposed.[128]

The definitions for healthcare-acquired infections in extended-care facilities[128] have also been applied to the home healthcare population.[129] Over 20% of patients were found to have an infection on the day of the survey; 5% of those infections had occurred during the course of home health care.[129]

5.1.3. Incidence Studies. Using definitions and methodologies outlined by the CDC for NNIS, a large number of incidence studies have been reported (Table 2). As noted previously, rates of nosocomial infections are most useful when reported for specifically defined populations at risk (e.g., bloodstream infections in patients with indwelling central venous catheters). In the most recent (2004) NNIS report, the rate of catheter-associated urinary tract infection (i.e., number of urinary catheter-associated urinary tract infections per 1,000 urinary catheter days) was 3.9 (10th–90th percentile = 1.3–7.9). This rate ranged from 3.0 in cardiothoracic ICUs to 6.7 in neurosurgical ICUs.[9] The rate of central venous catheter-associated bloodstream infections (i.e., number of central venous catheter-associated bloodstream infections per 1,000 catheter days) was 4.0 (10th–90th percentile = 1.7–7.6). This rate ranged from 2.7 in cardiothoracic ICUs to 7.4 in trauma ICUs.[9] Finally, the rate of ventilator-associated pneumonia (i.e., number of pneumonias per 1,000 ventilator days) was 5.4 (10th–90th percentile = 1.2–7.2). This rate ranged from 2.9 in pediatric ICUs to 15.2 in trauma ICUs.[9]

A 2006 study which investigated device-associated infections in 55 ICUs of 46 hospitals in eight countries noted all rates to be substantially higher than those previously reported in the United States.[130] The rate of catheter-associated urinary tract infection (i.e., number of urinary catheter-associated urinary tract infections per 1,000 urinary catheter days) was 8.9 (10th–90th percentilex = 1.7–12.8). The rate of central venous catheter-associated bloodstream infections (i.e., number of central venous catheter-associated bloodstream infections per 1,000 catheter days) was 12.5 (10th–90th percentile = 7.8–18.5). Finally, the rate of ventilator-associated pneumonia (i.e., number of pneumonias per 1,000 ventilator days) was 24.1 (10th–90th percentile = 10.0–52.7).[130]

5.1.4. Site-Pathogen Analysis. The site of nosocomial infection and the pathogen(s) reported as responsible

for them vary in incidence, relative frequency, morbidity, and mortality. The distribution of nosocomial infections by site varies by hospital service. This variation probably reflects differences in exposure to devices and procedures. CDC–NNIS data from 1996[131] report the site distribution of nosocomial infection based on the hospital-wide surveillance component as follows: urinary tract infection (27.2%), surgical site infection (18.7%), pneumonia (17.3%), primary bloodstream infection (15.8%), and other (21%). Site-pathogen data from the NNIS for the period 1990–1992 are given in Table 3.

5.1.4.1. Urinary Tract Infections. Infections of the urinary tract are consistently reported in Europe and the United States as comprising 30–45% of the total infections identified and are the most common cause of nosocomial infections.[4,24,105,132] Urinary tract infections are also the most common cause of infections in the long-term care facilities. The prevalence of UTI without indwelling catheters can be as high as 50% in women and 40% in men.[133] Over 60% of these infections are caused by gram-negative bacilli, with *Escherichia coli* causing approximately one quarter.[134] Enterococci and *P. aeruginosa* appear next in frequency in hospitals in the United States, while *Proteus* species are more frequently reported in the WHO data and in extended-care facilities in the United States. Secondary bacteremia appears in approximately 1% of individuals with nosocomial urinary tract infections, with the urinary tract being the second most common cause of healthcare-associated bloodstream infection. Increased morbidity and mortality independent of the infection's progression to sepsis have been noted in patients with healthcare-associated UTI.[135,136]

5.1.4.2. Surgical Site Infections. Surgical site infections are the second most common healthcare-associated infection in the United States accounting for approximately 20% of all nosocomial infections.[137] In some developing areas, up to one third of reported infections have involved surgical sites.[4] Approximately 500,000 SSIs occur annually, accounting for 3.7 million excess hospital days and over 1.6 billion dollars in excess hospital costs.[138] In hospitals participating in the NNIS program, 0.62 to 1.9% of patients with SSIs died from these infections.[105] Following a classification system for wounds (clean, clean contaminated, contaminated, or dirty) used in The National Research Council Study reported in 1964,[139] recent studies usually have reported risk of infection in postoperative surgical sites based on the probability of wound contamination during surgery. Surgical site infections usually arise as a result of intraoperative seeding of exogenous bacteria or as a result of dissemination of endogenous bacteria to the operative site. A number of factors determine the risk of surgical site infection, including the degree of microbiological contamination during the procedure, the duration of the procedure, and the patient's intrinsic risk. In order to use surgical site infection rates for comparative purposes, the rates should be adjusted

Table 3. Relative Frequency of Nosocomial Infection by Site and Selected Pathogens[a]

	Site (% of isolates)					
Pathogen	UTI	SSI	Pneumonia	BSI	Other	Percent
E. coli	25	8	4	8	4	12
S. aureus	2	19	20	16	17	12
Coagulase-negative staphylococci	4	14	2	31	14	11
Enterococci	16	12	2	9	5	10
P. aeruginosa	11	8	16	3	6	9
Candida spp.	10	4	6	8	6	7
Enterobacter spp.	5	7	11	4	4	6
Klebsiella spp.	8	4	10	5	4	6
Gram-positive anaerobes	0	1	0	1	19	4
Proteus mirabilis	5	3	2	1	2	3
Other *Streptococcus* spp.	1	3	1	4	2	2
Other fungi	3	0	1	1	1	2
Acinetobacter spp.	1	1	4	2	1	1
Serratia marcescens	1	1	3	1	1	1
Citrobacter spp.	2	1	1	1	1	1
Group B *Streptococcus*	1	1	1	2	1	1
Haemophilus influenzae	0	0	5	0	2	1
Other non-*Enterobacteriaceae* aerobes	0	1	4	1	2	1
Number of isolates	25,371	11,724	8,891	9,444	14,981	70,411

[a] Adapted from Emori and Gaynes[129]; NNIS 1990–1992.
No other pathogens accounted for more than 2% of isolates at any site.

for these risks. The NNIS has developed a surgical site infection risk index, which takes into account the patient's wound class, the American Society of Anesthesiology score, and the duration of the procedure.[123] In order to make comparisons between institutions or surgeons, surgical site infection rates should be stratified by risk categories.[140,141]

Staphylococcus aureus remains the most common isolate from wound infections,[4,21,105] although coagulase-negative staphylococci, enterococci, and gram-negative organisms have also been increasing in importance in recent years.[21] Only two-thirds of inpatients' surgical wounds and even fewer outpatients' wounds are cultured, despite clinical evidence for a surgical site infection.[142] It is important to note that a wound culture that grows a bacteria does not prove that a patient has a surgical site infection. Instead, the organisms may be colonizing the wound. Furthermore, a negative culture does not eliminate the diagnosis of a surgical site infection. For example, wound cellulitis often yields negative cultures and organisms such as *M. tuberculosis* are not detected by routine culture methods.

5.1.4.3. Respiratory Infections (i.e., Pneumonia). Nosocomial pneumonia is generally defined as pneumonia occurring 48 hours or more after hospital admission. Ventilator-associated pneumonia is a sub-group of nsoocomial pneumonia and is defined as pneumonia developing more than 48–72 hours after initiation of mechanical ventilation. Most published work on nosocomial pneumonia involves patients in intensive-care units, and in particular, mechanically ventilated patients who develop ventilator-associated pneumonia. Respiratory infections in the form of pneumonias make up a approximately 15–25% of all nosocomial infections. *P. aeruginosa* and *S. aureus* are the two most commonly reported pathogens.[21] Other gram-negative agents are also causal agents reported with significant frequency in most studies.[4,21,143] Published estimates suggest rates of 5–10 cases per 1,000 hospitalizations.[144,145] Patients with endotracheal tubes have rates that are up to 20-fold higher than patients without endotracheal tubes. A recent report showed that nearly 1 in 10 patients who required mechanical ventilation for more than 24 hours developed ventilator-associated pneumonia.[146] The chance of infection increases with duration of mechanical ventilation.

Nosocomial pneumonias are the leading cause of death from nosocomial infection. Secondary bacteremias are occur more frequently in nosocomial pneumonia than in either urinary or wound infections, and case-fatality rates for nosocomial pneumonias are only exceeded by those for bacteremia. Although the precise attributable mortality of nosocomial pneumonia is difficult to discern, some studies have suggested attributable mortality rates for ventilator-associated pneumonia as high as 30%. Multiple studies have shown that nosocomial pneumonia increases hospital length of stay by an average of 7–10 days. In patients with ventilator-associated pneumonia (VAP), the duration of both mechanical ventilation and ICU stay is increased, and the attributable cost of VAP at one tertiary-care medical center was nearly $12,000.[147] In another large epidemiologic study, patients who developed VAP had a mean increase of over $40,000 in hospital charges.[146]

5.1.4.4. Nosocomial Bloodstream Infection. Nosocomial bloodstream infections are one of the predominant causes of morbidity and mortality in the United States.[148] Central venous catheters are the source of most primary bloodstream infections,[149] leading to approximately 250,000–500,000 hospital-acquired bloodstream infections in the United States annually.[150–152] Since the 1980s, the incidence of primary healthcare-associated bloodstream infections has been rising steadily,[153] and the majority of these are related to the use of intravascular devices, especially central venous catheters.[154] Bacteremias remain the site with the highest case-fatality rate, exceeding 25% in many reports.[155–157] In addition, they cause a significant economic burden with a marginal cost estimate of $25,000 per episode.[151,158,159] Nosocomial bloodstream infections are reported as secondary to another site if there is documented infection at the primary site (in which case they are not included as an additional infection in determining rates) or primary if there is a culture-confirmed bacteremia without evidence for an underlying site of infection. Secondary bacteremias are documented in over 5% of infections at all sites and are most commonly associated with cardiovascular, intra-abdominal, and intravascular sites of primary infection. Infections with gram-negative organisms, including *Acinetobacter, Bacteroides*, and *Serratia*, have the highest rates of secondary bacteremias but are exceeded by far in relative frequency by *S. aureus* and coagulase-negative *Staphyloccus* infections.

Primary bacteremia rates are highest in teaching hospitals in the CDC–NNIS system, making up over 16% of infections in the larger teaching hospitals. The gram-positive organisms of coagulase-negative staphylococci and *S. aureus* have increased to become the most common agents of primary bacteremia in all but gynecology services where *E. coli* and *Bacteroides* species remain the most frequent. Recent data suggest that even among the general hospital population, bloodstream infections due to gram-negative organisms are again beginning to increase in number.[160]

5.1.4.5. Cutaneous Infection. Cutaneous infections make up approximately 6% of the infections reported from the NNIS system, with high rates in the larger university hospitals and on the newborn units. A larger portion of infections

in the developing countries and in extended-care facilities in the United States are reported at a cutaneous site.[122,124] *S. aureus* is the most frequently reported isolate at this site, with aerobic and anaerobic gram-negative rod organisms of increasing importance at this site in pressure ulcers common to the elderly in extended care.

5.1.4.6. Other Types of Nosocomial Infections. While the previously mentioned infections account for the clear majority of nosocomial infections, many other types of infection (e.g., gynecological, other upper respiratory, eye, gastrointestinal) may also occur in the healthcare setting.[79]

5.2. Epidemic Behavior and Contagiousness

Epidemic nosocomial infections represents only 5% of all nosocomial infections, yet they have been the most extensively reported. Epidemic behavior is determined by the causal bacteria, the route of transmission, and the characteristics of the host population at risk. These epidemics have mirrored advances in medicine just as the most common causal agents have moved from gram-positive streptococcal wound and puerperal infections, through resistant staphylococcal epidemics, to gram-negative bacilli of increasing antibiotic resistance infecting hosts with decreasing ability to resist.[161,162] In contrast, endemic nosocomial infections with gram-positive organisms have been increasing over the last decade compared to endemic gram-negative infections. Nosocomial epidemics investigated by the CDC have doubled each 5-year period since 1960.[163] During the period 1970–1975, 42% of the epidemics investigated were associated with some medical device and 70% were due to gram-negative bacilli.[164] From 1980 to 1985, 46.6% of the outbreaks investigated were related to products, procedures, or devices, and this proportion rose to 67.3% of investigations for the period 1986–1990.[162] Gram-negative bacteria accounted for 66% of the investigated outbreaks. Outbreaks of infection due to vancomycin-resistant enterococci have been increasingly reported in intensive-care units and oncology units, especially from large teaching hospitals.[165,166] More recently, outbreaks due to extended-spectrum beta-lactamase (ESBL)-producing Enterobactericeae as well as multidrug-resistant *P. aeruginosa* and acinetobacter species have been increasingly reported.[167–171] While this experience may represent unusually interesting or difficult outbreaks, it is not unusual for larger medical centers to experience several epidemics per year.

Many pseudoepidemics have been reported.[172–174] A pseudoepidemic is the occurrence of an increased number of isolates of a microorganism misinterpreted as a true epidemic of infection. The main factors in the psuedoepidemics have been improper specimen processing, surveillance artifact, and clinical misdiagnosis.[93]

5.3. Geographic Distribution

Nosocomial infections are not limited by geography (Table 2).[4] Lack of reported nosocomial infection has indicated inadequate study more frequently than an absence of risk. Service, organism, and extent of morbidity and mortality vary within and among different institutions. This variation frequently reflects differing case mix (severity of disease, comorbid factors) in the samples admitted from the population served. As a result, it is common for tertiary-care centers to exhibit higher rates than primary-care hospitals.[3,9] A direct relationship between rates of specific community-acquired infections and nosocomial risk has not been documented. However, the appearance of nosocomial epidemics as extensions of community-acquired and propagated epidemics has been recognized, as has the converse.[175] An excellent recent example of the impact of community infections propogated within the healthcare setting is the rapid emergence of community-associated MRSA.[176–178]

5.4. Temporal Distribution

An unexplained seasonal variation in certain gram-negative rods, including *Acinetobacter* with summer peaks, has been reported.[179,180] A recent study evaluated the association between weather patterns and occurrence of legionellosis, finding that cases of legionellosis occurred with striking summertime seasonality.[181] A significant dose–response relationship for occurrence of cases was seen with both precipitation and increased humidity. This finding is consistent with the current understanding of the ecological profile of legionella and supports the contention that sporadic disease due to this pathogen occurs through contamination of water sources.[181]

A direct correlation has been suggested between length of stay in a hospital and the incidence of nosocomial infection, but the confounding variables of disease severity, age, and comorbidity complicate this association. However, it is clear that the longer a person is hospitalized, the greater the likelihood exposure to possible risk factors for infection (e.g., indwelling devices, antimicrobial use, healthcare worker interactions).[182]

5.5. Age

Although persons ≥65 years of age comprised only 4% of the US population in the early 1900s, this group now

account for 13% of the total US population.[183] The number of US citizens living into extreme old age is growing exponentially with the most rapidly growing segment composed of individuals aged ≥85.[184] Currently, over 1.7 million people reside in over 17,000 long-term care facilities (LTCFs) in the United States, with this number expected to increase to 5 million by 2030.[185,186] Between 1.6 and 3.8 million infections occur in LTCF residents in the United States each year.[185,187–189] Extremes of age have been reported as directly associated with increasing nosocomial infection rates,[190] and the special problems in children[191] and in surgical site infection[192] have been recorded. Since extremes of age are known to be correlated with host defects resulting in increased susceptibility to infection, this hypothesis seems reasonable and attractive. Unfortunately, most studies have reported only relative frequency of infection by age group and have not been well controlled for length of exposure, disease severity, treatment, or other patient-related conditions that predispose to infection. Daschner et al.,[18] in a retrospective autopsy study, found an excess of nosocomial deaths in the 20–60-year-old age range. In a controlled study of risk factors for nosocomial infection in a university medical setting, age of 65 years or older increased the risk of nosocomial infection three times.[25]

Morbidity and mortality from nosocomial infection increase directly with age in the elderly, as for most infections, and the special problems of the neonate are similar to other infections in that age group.

5.6. Sex

Incidence of nosocomial infection has been reported to be greater in males than in females, but as with most other variables, these studies have not been well controlled.[157] Sex-related morbidity and mortality for nosocomial infection seem to parallel the site-pathogen experience for individual diseases.

5.7. Race

No race difference has been suggested or reported for nosocomial infections independent of other disease processes.

5.8. Occupation

Clinical healthcare professionals, medical laboratory workers, and other support personnel who come into contact with patients or patient specimens have potential increased risk of bacterial infection related to their employment.[193] Individual cases and outbreaks of a variety of bacterial infectious diseases (often *S. aureus* or MRSA) have been reported.[194–197] The most well-documented increased risk for clinical workers appears to be tuberculosis.[198,199] Medical laboratory personnel share this increased risk of tuberculosis and may have increased rates of brucellosis, shigellosis, *S. aureus*, and other enteric bacterial diseases.[197,200]

5.9. Occurrence in Different Settings

Healthcare-acquired infections have been reported in both acute- and extended-care settings. Within acute-care hospitals several high-risk units for nosocomial infection have been recognized. These include burn units,[198] high-risk nurseries,[191] intensive-care units,[161,201] dialysis units,[202,203] transplantation units,[204,205] and oncology units.[120] The common denominator in these units appears to be the use of specific hospital procedures and the presence of more severely compromised patients. The high nosocomial infection rates reported for university teaching hospitals may be directly related to the presence of these high-risk units. In one study of 38 US hospitals, 25% of all nosocomial infections reported occurred in intensive-care unit patients.[161]

Between 1.6 and 3.8 million infections occur in LTCF residents in the United States each year.[185, 187–189] Infections account for up to 50% of hospital transfers and 300,000 hospital admissions among LTCF residents annually.[206–208] Among LTCF residents, estimates of mortality from infection range as high as 388,000 deaths per year.[184,185,187,188] The annual cost of antibiotic therapy to treat infections in LTCF residents ranges from 38 to 137 million,[209,210] while costs of hospitalizations of LTCF residents due to infection range from 673 million to 2 billion each year.[209,210]

The proportion of medical care that is provided in the outpatient setting is increasing rapidly. For example, 80–90% of all cancer care is delivered in the outpatient setting and more than half of all hospital-based operations are done in ambulatory surgery centers.[211] From 1992 to 2001, patient visits to hospital outpatient departments increased from 56.6 million to 83.7 million visits. This represents a 48% increase, although the population in the United States increased by only 12% during the same time period. Office-based physician practices account for 80% of ambulatory healthcare delivered by non-Federal physicians, with 880.5 million patient visits in 2001.[212] While there is a recognized need for increased understanding of the epidemiology of nosocomial infections in this setting,[213] few data exist to date.

5.10. Socioeconomic Factors

While the importance of socioeconomic factors in infectious disease epidemiology is well recognized, no differential

has been reported for nosocomial infections independent of other disease processes.

5.11. Other Host Factors

In addition to age, the genetic and acquired modifications of host defenses usually important in increased susceptibility to infection contribute to nosocomial risk (Table 4). They include metabolic disease, cardiovascular disease, respiratory disease, cutaneous disease, and a spectrum of hematologic and immunologic abnormalities. Among the host defenses altered by these diseases are modifications in normal flora, changes in anatomical barriers to microorganisms, suppression of inflammatory response, and modifications in the reticuloendothelial system. Among the hematologic and immunologic factors important in nosocomial infection are those that decrease the number of neutrophils, cause defective function of neutrophils, reduce or cause defective immunoglobulin production, or interfere with cellular immunity. A single underlying condition may contribute to several defects in host defenses. A solid tumor may cause obstruction, erode a mucosal surface, and metastatically replace bone marrow. The resultant malnutrition, easy portal of entry, and loss of leukocyte function all contribute to infection by opportunistic microorganisms. The conditions that predispose to such opportunistic infections and the types of organisms involved have been well reviewed elsewhere.[214–216]

5.12. Treatment Factors

5.12.1. Antibiotics. Between 25 and 35% of hospitalized patients receive systemic antibiotics. Antibiotic treatment, including prophylactic antibiotic use, contributes to the risk of nosocomial infection by several means.[217] In addition to modifying the endogenous microflora of the host, several antibiotics interfere with immune function (chloramphenicol, rifampin) or contribute to renal (aminoglycosides), hepatic (erythromycin, isoniazid), or cutaneous (β-lactams) side effects that increase susceptibility to infection.

Under pressure of antibiotic use or abuse, the environmental microbial flora of medical care institutions increases in antibiotic resistance.[21, 113, 218–220] Patient acquisition of these resistant microbial flora begins shortly after admission and is accelerated by antibiotic treatment or prophylaxis, reaching as much as 60% of the patient population by the third week in some studies.[107] This acquisition is paralleled by an increase in the appearance of these bacteria in the causation of nosocomial infection (see Chapter 4).

5.12.2. Other Drugs. Those drugs with side effects that decrease normal host defenses have the most profound role in increasing risk of nosocomial infection. Corticosteroids modify inflammatory response, interfere with leukocyte function, and depress cellular and humoral immune responses. Antimetabolites and a variety of other cancer chemotherapy drugs have profound effects on all rapidly proliferating tissue, resulting in hemopoietic side effects that decrease immune function and gastrointestinal

Table 4. Examples of Factors that Modify Host Defenses

Host defenses	Disease modifiers	Treatment modifiers	Frequent sites of infection	Common organism of infection
Normal flora	Thermal burn	Antibiotic therapy	Soft tissue	*Pseudomonas*
		Gastric surgery	Intestine	*Salmonella*
Anatomical barriers	Trauma	Medical devices	Intravenous site	Coagulase-negative staphylococci
		Surgery	Wound	*S. aureus*
Mucociliary clearance	Cystic fibrosis	Irradiation	Pulmonary	*S. aureus*
		Tracheostomy	Pulmonary	*Pseudomonas*
Reticuloendothelial system	Sickle-cell disease, asplenia	Hemodialysis	Bloodstream	*S. pneumoniae* *H. influenzae* *Salmonella*
Leukocyte function	Chronic granulomatous disease, Job's syndrome, diabetic ketoacidosis	Corticosteroids Drug-induced neutropenia	Soft tissue Pneumonia	*S. aureus*
Humoral immunity	Agammaglobulinemia, chronic lymphocytic leukemia, multiple myeloma	Immunosuppression Cancer chemotherapy	Soft tissue Pulmonary	*H. influenzae* *S. pneumoniae* Gram-negative aerobes
Cell-mediated immunity	Thymic aplasia, malnutrition, Hodgkin's disease, HIV/AIDS	Immunosuppression Corticosteroids	Soft tissue	Gram-negative aerobes *Mycobacterium tuberculosis*

Table 5. Common Device-Related Nosocomial Infections[a]

Device[b]	Hospitalized patients with the device (%)	Most common related infection	Patients developing infection (%)	Case-fatality rate (%)
Urinary catheter	10	Bacteruria	20	<1
Intravascular infusion	12	Suppurative phlebitis	<0.01	20–40
Scalp vein needle	12	Suppurative phlebitis	<0.01	20–40
Plastic catheter	12	Suppurative phlebitis	0–8	20–40
TPN	0.1	Suppurative phlebitis	0–27	20–40
Arterial catheter	0.2	Suppurative phlebitis	0.2	20–40
Respiratory therapy				
Continuous ventilator	2	Pneumonia	—	40
IPPB	7	Pneumonia	—	40
Hemodialysis		Shunt infection	15–20	5

[a]Modified from Stamm.(164,221)
[b]TPN, total parenteral nutrition; IPPB, intermittent positive-pressure breathing.

side effects that interfere with secretory protection and cause loss of anatomical barriers through ulceration. The patient treated with a combination of intensive chemotherapy, immunosuppressive agents, and antibiotic prophylaxis in an attempt to induce remission of leukemia or in preparation for bone marrow or organ transplantation epitomizes the host at special high risk of nosocomial infection secondary to drug effects.

5.12.3. Device- and Procedure-Related Infections. Medical implements and devices and the procedures associated with them have assumed a common necessary part of modern medical practice. These devices include intravenous catheters, drains, urinary catheters, indwelling tubes placed into the normally closed body cavities of the peritoneum or the cardiothoracic spaces, prosthesis in the cardiovascular system, the central nervous system, or the skeletal system, as well as a variety of implements in the respiratory system, including nasotracheal, endotracheal, and tracheostomy tubes. Each of these devices provides a route of entry for microorganisms by penetrating host defense mechanisms and acts as inanimate foci for the proliferation of microorganisms. One or another of the devices is used in the care of approximately 10% of patients hospitalized in acute-care facilities. It has been estimated that 45% of all nosocomial infections, over 850,000 per year in the United States, are device related (Table 5).(9,164,221)

6. Mechanisms and Routes of Transmission

Patients may be infected with bacteria acquired from either endogenous or exogenous sources. Microorganisms from endogenous sources may appear by reactivation of some previous infection, as in tuberculosis, or by invasion of commensal flora, which infect because of some reduction in host defenses. Transmission from exogenous sources may take place by hands, by the airborne route, through fomites, by insects, or by ingestion of contaminated food or water. While each of these routes, with the exception of insects, has been described in individual outbreaks, transmission by direct and indirect contact remains the common and most significant route in acquiring nosocomial infection. Exogenous sources are found in three specific reservoirs: (1) within the fixed structures of the facility itself; (2) in devices or equipment used within the institution; and (3) in medical personnel, other patients, or visitors.

6.1. Inanimate Reservoirs

The microenvironment of the hospital, rich in water and nutrient sources, has provided a continuing niche for gram-negative bacilli that have evolved as important agents in nosocomial infection. *Enterobacter, Serratia, Acinetobacter, Citrobacter, Flavobacteria, Legionella*, and *Pseudomonas* species have been repeatedly identified as important nosocomial pathogens because of their ability to survive in the aqueous and other fluid reservoirs of our institutions.(167,222–225) Clean environmental surfaces are generally not important sources of bacteria-causing nosocomial infections.(68) However, environmental surfaces contaminated with *Clostridium difficile* or vancomycin-resistant enterococci have been implicated in the nosocomial transmission of these organisims.(226,227) Extensive reviews of reported inanimate reservoirs are available.(225,228)

6.1.1. Structural or Engineering Reservoirs. The medical care field is one of rapid change, and this is evident in frequent requirements for modification of structures used for medical care. With limited funding, compromises in terms of structure, space, and function that are less than ideal may be accepted. The result may be crowding,

which encourages cross-contamination, or the inconvenient placement of certain fixed equipment (e.g., hand-washing sinks), which makes the use of the equipment problematical. Construction or reconstruction has also been associated with increased rates of nosocomial fungal infection. Furthermore, reconstruction may disguise an inadequate air-conditioning system, resulting in the recirculation of unfiltered air from an "isolated" tuberculosis patient[193] or of air exhausted from microbiological laboratories or other areas of high biohazard.

Legionella pneumophila is ubiquitous in water and may be present in potable water. Nosocomial Legionnaires' disease has been described as resulting from contaminated cooling towers of air-conditioning systems and potable water as well as from construction activity in the vicinity of the hospital.[229–232] Reservoirs of nosocomial bacteria within water supplies or plumbing have been a recurrent problem in hospitals caring for high-risk patients.[222,223,233]

6.1.2. Equipment Reservoirs. In addition to the common medical devices, a wealth of new and increasingly complex equipment attends patient care. Respiratory assistance equipment, cardiovascular assistance pumps, microelectronic monitoring devices, dialysis machinery, and numerous other "black boxes" have reached the bedside. Appropriate use of these instruments is understood by fewer and fewer individuals, and the safe techniques for appropriate disinfection between patients may be overlooked or misunderstood. Portions of this equipment may be expensive, fragile, nondisposable, and unable to withstand the usual harsh but sure techniques of heat sterilization. Alternate methods are often less well understood than classic heat sterilization, and inadequate attention is paid to the detail necessary to assure their full implementation. As a result, residual microorganisms may be transmitted to subsequent patients as has occurred with a variety of devices, including bronchoscopy and endoscopy equipments.[219,234–236] Other problems have arisen when bacterial endotoxins have remained on equipment, causing severe reactions, or residual chemical sterilants themselves have caused toxic effects.[237]

6.2. Animate Reservoirs

Bacteria causing nosocomial infection may reside in the animate reservoirs[109,164,238–242] of the healthcare worker or of the chronically institutionalized patient. Patients colonized with nosocomial organisms at clinical sites, such as wounds or urine, or colonized in their gastrointestinal or genitourinary tract, or their oropharyngeal flora serve as reservoirs for these organisms.[112,243,244] Hospital personnel are occasionally personal carriers of nosocomial organisms such as *Salmonella*, staphylococci, or enterococci that are transmitted to hospitalized patients. Of these characteristics, the ability to survive at the time of transmission in mechanical carriage remains the most significant factor in nosocomial infection.

Hospital professionals, medical support workers, and patients provide the animate reservoirs for microorganisms. Biological transmission through symptomatic or asymptomatic carriage of bacteria has led to serious and extensive epidemics.[163,239] While visitors are a potential for microorganisms, they have rarely been implicated in such transmission.

Even more commonly, healthy but careless individuals mechanically transmit large numbers of bacteria from reservoirs within the facility, from equipment, and from patient to patient during their daily rounds.[245] Hand carriage of microorganisms is recognized as the most important factor in endemic and most epidemic spread of nosocomial infection. Multiple investigations conducted during the last 40 years have confirmed the important role that contaminated hands of healthcare workers play in transmission of healthcare-associated pathogens.[246] As a result, hand washing has for many years been considered one of the most important measures for preventing the spread of pathogens in healthcare settings.[247] In one study in a university neurosurgical unit, 44% of randomly sampled personnel carried gram-negative bacilli and 11% had *S. aureus* on their hands.[248] More important, serial culturing demonstrated that all persons at various times carried gram-negative bacilli and two-thirds carried staphylococci. Indirect contact spread via transient hand carriage has been implicated in other nosocomial transmission of staphylococci, enterococci, and gram-negatives.[109,238,240,242,249] Such carriage is instrumental in the nosocomial colonization that precedes infection in the compromised host.[106,107,241,250,251]

7. Pathogenesis and Immunity

The pathogenesis of nosocomial infection is determined by host and environmental factors that are uniquely combined in healthcare institutions.

The susceptible patient is prey to all the conventional pathogens. The compromised host is infected by a much smaller dose of bacteria and by species that are usually considered commensal and "nonpathogenic."[214] Thus, the host with underlying severe disease and impaired host defenses has higher rates of infection, secondary bacteremia, refractory infection, and case fatality.[17,252] As in the example of hematologic cancers, disease and treatment have so devastated the immune system that response is lethargic or nonexistent, infection is poorly controlled, even by

appropriate antibiotic therapy, and case mortality is high.[253] Following infection with agents that persist in endogenous sites (e.g., *M. tuberculosis*), significant immune depression may result in reactivation and uncontrolled dissemination.

Pathogenicity by nosocomial agents has not been related to the presence of resistant bacteria.[254,255] However, antimicrobial resistance has been frequently associated with worse clinical outcomes.[10] One possible explanation is noted in recent studies focusing on bloodstream and respiratory tract infections in hospitalized patients which suggest that a delay in adequate antimicrobial therapy results in significantly poorer outcomes, even when controlling for other important predictors of mortality.[55,56,256–259] One common reason for prolonged time to adequate therapy is that choices for empiric therapy did not ultimately match the organism identified (often due to infection with a resistant pathogen).[53,55]

The selective pressure of antibiotic use assures the presence of resistant forms available for transmission in the healthcare setting.[243] At each site, colonization precedes local multiplication and tissue infection.[106,260,110] Colonization with nosocomial organisms results from alterations in "normal flora" by broad-spectrum therapy, hospitalization, and instrumentation. Colonization is directly related to the organisms' survival ability, ability to adhere to mucosal surfaces and catheters, and organism virulence. Special procedures and devices allow bacteria to be delivered past the superficial barriers and to sustain a milieu important to implantation and multiplication.

8. Patterns of Host Response: Clinical Features and Diagnosis

Patterns of host response to nosocomial infection are those classically described with bacterial disease of the sites involved. As with pathogenesis, clinical features and morbidity are determined by the virulence of the agent, the dose and route of transmission, and the character of the host defense mechanisms.

Signs and symptoms in the compromised host may be altered or reduced. Fever may be low or absent. The site of infection may be unusual, as in the predilection to perirectal abscess in cancer patients.[261]

8.1. Urinary Tract Infection

Infections of the urinary tract account for one third of all nosocomial infections.[4,24,105,132] Urinary tract infections are also the most common cause of infections in the long-term care facilities. *E. coli, E. faecalis*, and *P. aeruginosa* are the most common causal organisms.[21] The great majority of healthcare-associated UTIs result from instrumentation of the urinary tract, usually catheterization. Catheterization is a common practice; approximately 15–25% of hospital patients have a urinary catheter at some time during their stay, with rates of utilization varying by unit type within the hospital.[262] Indwelling urinary catheters are also very common in LTCF residents.[133] The risk of contracting catheter-associated UTI increases with the duration of catheterization.[263] Females, the elderly, and persons with severe underlying illness are also at increased risk of healthcare-acquired urinary tract infection.[236] The overall risk of bacteriuria in hospitalized patients with indwelling catheters is about 25%. Bacteria gain access to the catheterized bladder by either of two routes. They may migrate from the collection bag or the catheter drainage tube junction, or ascend extraluminally within the periurethral space.[260] Bacteria can adhere to and grow on the inner surface of the catheter, leading to a biofilm that also includes urinary proteins and salts. Bacteria embedded in this biofilm are then less susceptible to antibiotics.[264,265] Although urinary tract infection is typically characterized by dysuria, frequent and/or urgent urination, bacteriuria, and pyuria,[266] these findings may be attenuated or absent in the elderly or in immunocompromised patients. Greater than 10^5 colony-forming units per milliliter of urine represent significant bacteriuria from a clean-catch midstream urine specimen. In catheterized patients, however, a concentration of microorganisms considerably less than 10^5 can be shown to increase if untreated and can be associated with symptomatic infection.[267] The significance of asymptomatic bacteriuria in patients with indwelling bladder catheters remains under discussion.[135,268] Recent guidelines concerning asymptomatic bacteriuria from the Infectious Diseases Society of America suggest that urine cultures not be obtained in asymptomatic catheterized patients.[269] Furthermore, the goal of therapy of asymptomatic catheter-associated bacteriuria remains poorly defined with bacteriuria recurring shortly after cessation of antimicrobial therapy.[269]

8.2. Nosocomial Pneumonia

Pneumonia appears frequently in patients in whom aspiration has taken place or in whom the upper airway is bypassed by endotracheal intubation. The diagnosis of nosocomial pneumonia is difficult to make and often relies on the combination of clinical criteria and microbiological data.

Purulent sputum, pulmonary infiltration on X ray, fever, and hypoxia may indicate the presence of this complication. In individuals with community-acquired pneumonia, a change in microbial flora in the sputum accompanied by a worsening in clinical course usually indicates a pulmonary superinfection. Culture and Gram's or other staining of the sputum are important in establishing the etiologic diagnosis.[86,143,270] Protected specimen brush samples or bronchoalveolar lavage is important in more clearly defining the microbial etiology of nosocomial pneumonia.[145] Organisms simultaneously isolated from the blood and pleural fluid are strong evidence of pulmonary infection with that agent.

8.3. Surgical Site Infection

Infection of the surgical site is suspected in any febrile postoperative patient. Drainage of purulent material or disruption of the wound is considered prima facie evidence of wound infection regardless of the presence or absence of bacterial isolates. Unfortunately, up to 60% of surgical site infections are not evident until after the patient is discharged from the hospital which makes tracking of these infections difficult.[140] Infection following certain procedures such as implantation of a prosthesis or other foreign material may only become evident after months.

8.4. Bacteremia

Bacteremia is usually accompanied by fever and a decline in clinical condition. In the absence of evidence of infection at other sites, an intravenous source of infection should be suspected. Intravascular catheters in place for more than 48 hours should be investigated, even in the absence of inflammation.[271] Blood cultures, cultures of infusates, and semiquantitative cultures of the catheter itself may establish the diagnosis.[272] A recent meta-analysis noted that paired quantitative blood culture is the most accurate test for diagnosis of central venous catheter-related bloodstream infection.[273] However, most other methods studied showed acceptable sensitivity and specificity and negative predictive value. The positive predictive value of all tests increased with high pretest clinical probability.[273]

8.5. Infection at Other Sites

Nosocomial infection of the skin, soft tissue, gastrointestinal tract, urogenital tract, and other sites may be found. More detailed discussion of the appearance, diagnosis, and treatment of these infections has been published.[274,275]

9. Control and Prevention

9.1. General Concepts

The methods for conducting a successful nosocomial infection control program have been recently reviewed[78] (Table 6). Identification of nosocomial infections through application of standard criteria is widely accepted.[9] Routine, continuous collection, collation, evaluation, and reporting of these data by a trained specialist using "surveillance" tools are recommended and have been tested for efficacy by the CDC during the SENIC Project[28] (see Chapter 2). The presence of the infection control professional (ICP) on the clinical unit is also of value in (1) reinforcing the importance of infection control measures; (2) providing early identification of epidemics; (3) providing informal, concurrent, in-service education; and (4) assisting in providing new information for research efforts in the area of nosocomial risk control. The presence of one ICP for approximately every 250 inpatient beds is important to efficacy. However, it has been noted that this ratio is no longer adequate as the amount and complexity of the work of the ICP has increased, the care of patients in the healthcare setting has become more complex, and the severity of illness of the hospitalized patient population has continued to increase.[78] The presence of a trained hospital epidemiologist is also of great value.[78] Evaluation of institutional nosocomial infection data through these techniques has allowed identification of priority infection problems and planning, implementation, and evaluation of interventions to control them. Control programs usually fall into three areas: preventive engineering, equipment control, and personnel programs.

9.1.1. Preventive Engineering. Infection control personnel must collaborate with engineers, nurse managers, administrators, architects, and physicians before, during, and after construction or reconstruction projects. Various

Table 6. Elements of Nosocomial Infection Control

1. Elaborate criteria for infection
2. Establish surveillance
3. Determine endemic rates
4. Evaluate endemic problems
5. Recognize and control epidemic
6. Effect control program
a. Orientation
b. In-service education
c. Behavior modification
7. Evaluate control programs
8. Evaluate cost–benefit

guidelines have been published by professional societies as well as state and national agencies.[276–278] Efficient function follows appropriate form in the hospital as in other industries. If hand-washing sinks are present and conveniently placed in patient care areas, it is more likely that they will be used. Traffic flow will be determined by design and will be difficult to alter by placing signs thereafter. Control of environmental bacterial contamination will be affected by surface features, air-conditioning, and the convenient presence of housekeeping and other service modules.

9.1.2. Equipment Control. As with structure, the design of equipment is important to its correct use, maintenance, and disinfection. Initially inexpensive devices may multiply future costs by requiring complicated disassembly, cleaning, and time-consuming disinfection in order to render them safe for multiple patient use. Component parts may not tolerate heat sterilization and may require gas or chemical sterilization. Gas and chemical sterilizations are more demanding in detail and may leave residuals that present toxic problems.[237] The use of disposable equipment has exploded in the hospital industry, contributing to major increases in cost and in problems of waste disposal. Reuse of disposable equipment on individual patients, under strict protocols, has been found acceptable in a few limited situations (e.g., hemodialyzer reuse).[270,279,280] However, attempts to modify this expense by reuse of disposable equipment on multiple patients have been responsible for several outbreaks of nosocomial infection and are to be decried.[281] Intrinsic contamination of industrially prepared medical devices and supplies has been a rare cause of epidemic nosocomial outbreaks and has usually been associated with and identified by the unique bacteria involved.[248,282]

9.1.3. Personnel Programs. Healthcare personnel are the common final factor in the transmission of most nosocomial infections. Orientation and in-service education and reeducation programs are important in gaining staff appreciation of infection control programs and techniques. The direct availability of the ICP during on-the-ward surveillance activities provides important one-on-one education and consultation in support of bedside infection control. Behavior modification through management techniques of observation, retraining, and the use of incentives and penalties is recommended and under expanding use as effective infection control efforts.[283] Occupational health programs, which assure that healthcare workers are screened and vaccinated as indicated, for transmissible diseases on employment and have the opportunity to have subsequent illnesses evaluated for potential transmission, are also an important feature of control of animate reservoirs. [284–286]

9.2. Special Control Programs

9.2.1. Hand Washing. Routine hand washing before, between, and after contact with patients is recognized as the most important feature of successful infection control.[78,246,247] The use of an antiseptic soap is recommended in high-risk areas and when vancomycin-resistant enterococcus is isolated,[227] but otherwise is of little documented improvement over the routine use of a vigorous scrub with soap and running water. The use of gloves does not replace the need for hand washing. In a recent study involving 2,800 observed opportunities for hand hygiene in a large hospital, factors associated with poor compliance with hand hygiene included a high intensity of care (i.e., many opportunities for hand hygiene per hour of patient care), weekdays, working in an intensive-care unit (ICU), and procedures associated with a high risk of contamination.[287] Poor adherence associated with a high intensity of care suggested that HCWs did not have enough time to wash their hands as frequently as recommended.[287] Alcohol-based hand rubs (gels, rinses, foam) have characteristics that address several problems associated with washing hands with soap and water. Alcohol solutions reduce bacterial counts of the hands of volunteers to a greater degree that washing hands with nonantimicrobial soap and water, and antimicrobial soap and water.[246] Also, because alcohol-based hand rubs do not require the use of water, they can be made available at many more locations than hand-washing facilities. Finally, making such products readily accessible (near each patient's bedside or in pocket-sized bottles) has been shown to improve adherence of HCWs to recommended hand hygiene practices.[288] In ICUs, where the workload is high, with corresponding elevated numbers of indications for hand hygiene per hour of patient care,[287] preferential use of handrubs can reduce the impact of time constraints, with subsequent improvement in compliance.[289] Extensive reviews of hand-washing procedures and guidelines have been published.[33,67,245,246,290]

9.2.2. Standard Precautions and Isolation Control. The goal of isolation is to prevent transmission of microorganisms from infected or colonized patients to other patients, hospital visitors, and healthcare workers. Personal protective equipment (e.g., masks, eyewear, gloves, and gowns) and specific room requirements are critical in accomplishing this goal. Appropriate isolation remains the cornerstone of infection control and is assuming greater importance as the prevalence of multiply antibiotic-resistant organisms increases.

Techniques for the recognition and appropriate care of patients with potentially communicable disease have been recommended in guidelines published by the CDC.[291] The recognized inability to identify accurately all such individuals on admission and the potential for healthcare worker

exposure to HIV and other blood-borne disease(s) have led to the recommendation that a modified method of care be used for all patients with whom there is expected to be contact with blood or body fluids. This system of "universal precautions" recommends the use of gloves in any patient care situation where such contact is expected and the additional use of gown, mask, and eye protection where splashing, aerosolization, or more extensive exposure is common.[291]

In the CDC guideline, there are two tiers of precautions.[291] The first tier is referred to as *standard* precautions and is to be used for the care of all patients, regardless of diagnosis. Standard precautions incorporate the major features of both universal precautions and body substance isolation and are designed to reduce the risk of transmission of organisms, including blood-borne pathogens, to patients and healthcare workers. The second tier, referred to as *transmission-based* precautions, includes airborne precautions, droplet precautions, and contact precautions. These precautions are designed for the care of specific patients infected or colonized with epidemiologically significant organisms that are spread by the airborne or droplet route or by contact with dry skin or contaminated environmental surfaces. These precautions are to be used in addition the standard precautions.

9.2.3. Epidemic Investigation and Control. The routine collection of surveillance data on nosocomial infections allows one to establish endemic rates by type of nosocomial infection, by organism, and by area and service within the medical facility. This allows rapid recognition of unusual or epidemic events, whether through the appearance of a new or highly resistant microorganism or through a grouping of similar infections. Advances in information technology has allowed a more systematic and streamlined approach to establishing baseline rates of infection and setting triggers for evaluation of a possible outbreak.[35,36] An outline of an epidemic investigation is given in Table 7. The careful documentation and reporting of epidemic investigations are important features in every infection control program.[292]

9.2.4. Nosocomial Urinary Tract Infection. The vast majority of documented nosocomial urinary tract infections are associated with catheter use. The use of a closed sterile drainage system with indwelling catheters provides a significant (50%) short-term reduction in infection related to this device.[293] Appropriate care in the placement and maintenance of these devices is essential to their success in preventing nosocomial infection.[260,294] Breaks in aseptic technique in placement and disruption and entry into the closed system are the most significant factors in the development of catheter-related urinary tract infection.[295] Despite the clear risk, catheters are often overutilized and unnecessary, placing patients at needless risk of contracting infection.[263] Better methods of monitoring catheter utilization and reducing the inappropriate use of urinary catheters translate into reduced rates of healthcare-associated UTIs.[296] Guidelines for the control of nosocomial urinary tract infection have been developed and published by the CDC.[297] An outline for catheter use and care is seen in Table 8.[298] In the SENIC project analysis, the combination of high levels of surveillance activity, a modest level of control activity, and the presence of a full-time ICP yielded an estimated 31% reduction of infection in patients at high risk for this event.[299] Asymptomatic bacteriuria is a common finding and the latest IDSA guidelines recommend that cultures be performed in pregnant women and individuals about to undergo a urologic procedure.[269] Screening cultures in asymptomatic individuals should not be performed.[269] Recent attention has also been paid to the role of urinary catheters coated with antimicrobial substances. A recent systematic review summarized the results of studies performed to date evaluating nitrofurazone coated or silver alloy coated urinary catheters.[294] Both types of antimicrobial-coated catheters can prevent bacteriuria during short-term catheterization in hospitalized patients. Evalution of the impact of coated catheters on symptomatic UTI was not assessed in any of the reviewed studies.[294]

9.2.5. Nosocomial Surgical Site Infection. Nosocomial surgical site infections are usually initiated at the time

Table 7. Outline of Epidemic Investigation[a]

1. Establish the existence of an epidemic
2. Complete case count
3. Develop epidemiologic data
4. Form hypothesis
5. Test hypothesis
6. Institute control measures
7. Evaluate control measures
8. Document and report

[a]Modified from a CDC training pamphlet.[178]

Table 8. Guidelines for Urinary Catheter Use[a]

1. Do not use unless necessary
2. Use aseptic techniques
3. Use sterile-closed drainage system
4. Maintain system closed
5. Keep bag below level of bladder, but not on floor
6. Culture aseptically
7. Discontinue early
8. Sanitize measuring devices after each use

[a]Modified from Hustinx and Verbrugh.[182]

of surgery.[300–302] The responsible microorganisms originate from the patient's endogenous flora, from operating room personnel, or from the operating room environment. Guidelines featuring the etiology and control of surgical site infections have been published by the CDC.[141,279] Reporting SSI rates to practicing surgeons has been shown to reduce rates, likely due to having one's behavior observed. However, one must interpret the conclusions with caution as most studies have included confounding factors.

One must ensure that best practices are followed for proper hair removal and antiseptic skin preparation. Patients who are colonized with *S. aureus* in the anterior nares are at increased risk of the development of surgical site infection as well as other nosocomial infections.[303] A recent randomized double-blind clinical trial demonstrated that treatment with preoperative mupirocin decolonized the nares of patients and decreased *S. aureus* nosocomial infections in only a subset of patients who were colonized.[303] Treated patients also had decreased *S. aureus* SSI, but the decrease was not statistically significant. Further study is needed to determine if this intervention can benefit a targeted subset of surgical patients. Of note, a recent study noted that routine culture for *S. aureus* nasal carriage at admission and subsequent mupirocin application did not provide effective prophylaxis against nosocomial *S. aureus* infections in nonsurgical patients.[304]

The routine use of prophylactic antibiotics just prior to selected surgical procedures has been well studied, and the practice is supported by an ever growing number of investigations. Prophylactic antibiotics are meant to decrease microbial proliferation at the incision site. The timing of prophylactic antibiotics is crucial in determining efficacy in preventing surgical site infections. The optimum time for administration has been shown to be during the 1 hour period before the incision.[305] Antibiotic prophylaxis is recommended for clean-contaminated procedures and clean procedures involving the implantation of a foreign body. Several reviews of these recommendations have been published.[306–308] General guidelines for the approach to surgical prophylaxis are reviewed in Table 9. Recent studies have shown that there is much room for improvement in the administration of antimicrobial prophylaxis.[305] Indeed, the Centers for Medicare and Medicaid Services (CMS) has made proper administration of preoperative antibiotics, a quality performance standard for hosptials.

Proper ventilation should be maintained in all operating rooms, including positive-pressure and appropriate air filters. In addition, traffic through the operating room should be kept to a minimum. Normothermia should be maintained during surgery as studies have demonstrated that intraoperative hypothermia approximately triples the risk of surgical site infection and leads to many other adverse events.[309] Likewise, hyperglycemia increases the likelihood of surgical site infection.[310] Thus, interventions to screen for diabetes and hyperglycemia with administration of intensive insulin therapy should be considered.

Table 9. Guidelines for Perioperative Antibiotic Prophylaxis[a]

1. Clean-contaminated procedure or clean procedure with foreign body
2. Antibiotic spectrum effective
3. Effective concentrations of antibiotic present at incision time
4. Use single-dose regimen—additional intraoperative dose for lengthy procedure
5. Use low-toxicity regimen
6. Avoid antibiotic critical to therapy
7. Benefits should outweigh risk

[a]Modified from Page et al.[193]

9.2.6. Nosocomial Respiratory Infections. Preventive strategies to lower the incidence of nosocomial pneumonia focus on reducing or eliminating risk factors for oropharyngeal or gastric colonization and subsequent aspiration of contaminated oropharyngeal or gastric secretions. Recent guidelines for prevention of healthcare-associated pneumonia have been published.[311] Data from the SENIC project suggest that a high level of surveillance and the presence of an ICP were a necessary feature for the 27% reduction in postoperative pneumonia reported. Reductions in medical pneumonia appeared much less successful.

Endotracheal intubation is one of the most important risk factors contributing to pneumonia in patients requiring mechanical support for respiratory failure. Increasingly, the use of non-invasive or positive-pressure mask ventilation in selected groups of patients has been shown to be effective in reducing the need for endotracheal intubation.[312] While not appropriate for all patients, the use of non-invasive ventilation in selected populations appears safe and may reduce the likelihood of nosocomial pneumonia.[313]

Supine patient positioning may contribute to the development of ventilator-associated pneumonia, likely due to an increased risk of gastric reflux and subsequent aspiration. Several studies have demonstrated a benefit of semi-recumbent positioning (elevation of the head of the bed $>45^{\circ}$) in reducing ventilator-associated pneumonia.[314] Generally, patients were excluded from these studies if they could not tolerate an upright position due to hemodynamic instability or recent surgery. Furthermore, few data exists on non-ventilated patients.

Medications that increase gastric pH, such as H2 antagonists and antacids, allow for colonization of the upper gastrointestinal (GI) tract by potentially pathogenic organisms and may increase the risk for nosocomial pneumonia.

Sucralfate, an agent often used for stress ulcer prophylaxis, has limited effect on gastric pH and therefore may not increase the risk for pneumonia. Seven meta-analyses of over 20 randomized trials have investigated the impact of stress ulcer prophylaxis on risk of nosocomial pnuemonia in critically ill patients.[315] Four showed significant reductions in the incidence of pneumonia and three showed similar but non-significant trends toward reduced pneumonia in patients on sucralfate. A recent randomized trial, however, compared sucralfate to H2-antagonists and found no effect on pneumonia, but a significant increase in the risk of clinically important bleeding with the use of sucralfate.[316] This issue, thus, remains controversial with a priority being the identification of patients most likely to benefit from stress ulcer prophylaxis.

Selective digestive decontamination refers to sterilization of the oropharynx and GI tract in mechanically ventilated patients in hopes of preventing the aspiration of large numbers of organisms and subsequent pneumonia. Most evaluations of selective digestive decontamination have involved topical agents such as polymixin, aminoglycoside, and amphotericin. In some studies, investigators have also added short course IV therapy. There have been seven meta-analyses of over 40 randomized trials of selective digestive decontamination which have been recently summarized in a systematic review.[315] All seven trials showed significant reduction in the risk of ventilator-associated pneumonia with the use of SDD. However, it is unclear how routine use of selective digestive decontamination might affect antibiotic resistance patterns. While selective digestive decontamination is utilized more commonly in Europe, its use in the United States is limited primarily due to concerns about its possible effect on emerging antimicrobial resistance.[317] While potentially promising as a preventive measure, further study with careful evaluation of the impact of this strategy on antibiotic resistance is warranted.

9.2.7. Nosocomial Intravascular Infection. An accurate and practical surveillance system is essential for identifying the true scope of the problem and thereby allowing for implementation of effective strategies to reduce catheter-related bloodstream infections. Surveillance can provide the basis for an effective infection control program through the routine and orderly collection of data, subsequent analysis and timely reporting. In the SENIC study, hospitals with a high-intensity control program, regardless of the level of surveillance activities, showed a moderate 15% reduction in nosocomial bacteremia rates.[28] However, hospitals with a similar intensity control program but with at least a medium level of surveillance demonstrated a 35% reduction in nosocomial bacteremia rates. Proper choice of device, correct placement, and careful maintenance of intravenous systems are important in the control of infections at this site.[318] Care in prevention of contamination of the delivery system is important as is the documentation of the appropriate use of additives through dating and labeling. Recent guidelines for the prevention of nosocomial intravascular infections have been prepared by the CDC.[319] A recent study evaluated the impact of an evidence-based strategy including various infection control interventions (hand washing, using full-barrier precautions during the insertion of central venous catheters, cleaning the skin with chlorhexidine, avoiding the femoral site if possible, and removing unnecessary catheters to reduce intravascular infections).[320] This bundled intervention resulted in a large and sustained reduction (up to 66%) in rates of catheter-related bloodstream infection that was maintained throughout the 1.5-year study period.[320] Success in BSI reduction has been found with antibiotic-impregnated and antiseptic (e.g., chlorhexidine/silver-sulfadiazine)-impregnated catheters in several randomized trials.[321,322] However, conflicting data, questionable cost-effectiveness, and the potential for adverse events (e.g., anaphylaxis, emergence of drug resistance) have limited the widespread use of these novel devices. Current guidelines state that antibiotic/antiseptic-impregnated catheters should be used if the rate of infection is high despite adherence to other strategies.[323]

Important features for intravenous care are outlined in Table 10.

9.2.8. Antimicrobial Prophylaxis. The routine prophylactic use of antibiotics has not been demonstrated to control nosocomial infections in most sites and situations. The use of prophylactic antibiotics for surgical procedures is discussed in Section 9.2.5. Although prophylactic antibiotics, in the form of selective digestive decontamination, have been

Table 10. Guidelines for Intravenous Device Use[a]

1. Do not use unnecessarily
2. Choose a low-risk site
3. Use good technique
   a. Wash hands
   b. Full drapes for insertion
   c. Use aseptic prep and fixation
   d. Date site
   e. Label appropriately
4. Maintain appropriately:
   a. Discontinue emergent site early
   b. Observe daily
   c. Change set not more frequently than every 72 hour
5. Remove early
6. With sepsis, suspect iv site

[a]Modified from CDC guidelines.[200]

studied in the prevention of nosocomial pneumonia (Section 9.2.6), this practice remains controversial.

9.2.9. Immunization. Immunization for the prevention of bacterial nosocomial infection has not been exploited, largely due to the lack of effective vaccines. However, in other institutional situations pneumococcal and meningococcal vaccines have been tested and found successful. Both passive and active immunizations for control of nosocomial *Pseudomonas* infections in oncology and burn units have been investigated, but remain investigational.[324,325] Development of a vaccine against nosocomial *S. aureus* is also underway.[326]

9.2.10. Cellular and Transplantation Methods. Granulocyte transfusions have improved the response to infection in some leukemia patients with increased survival. This technique is limited by lack of sources for sufficient cells and recurrent concern about the ability to ensure their safe use in excluding occult microbial agents. Bone marrow transplantation is an increasingly successful technique, but one that requires great sophistication and resources. Hematopoietic growth factors, such as granulocyte and granulocyte–macrophage colony-stimulating factors, are increasingly being used in neutropenic patients. These growth factors stimulate the bone marrow to produce neutrophils, thereby shortening the duration of neutropenia following chemotherapy.

10. Unresolved Problems

10.1. Efficacy and Cost–Benefit Evaluation: Acute Care

The program of surveillance and infection control recommended by the CDC and mandated by governmental and accreditation agencies has benefited from the findings of the SENIC project.[28] The results showed a 32% overall reduction in nosocomial infection through application of a systematic infection control program. Since the data on which the study was based were gathered in 1970 and 1975–1976, the applicability to hospital practice two decades later remains open to some question. Medical care continues to change at a rapid pace with new technologies and shifts in practice, which have markedly altered the case mix and intensity of services offered in acute hospital care in the United States. As a result, an even higher portion of nosocomial infections may not be preventable by classic CDC methods, especially those from endogenous sources in highly immunocompromised patients.[261] Analyses have demonstrated nosocomial infection prevention and control programs to be not only clinically effective but also cost-effective,[78] particularly given the high cost of preventable nosocomial infections. Although continued studies evaluating the contemporary cost-effectiveness of infection control programs are needed, these programs appear well justified in the competition for resources in acute medical care.

10.2. Coordinated Programs

The many common features in nosocomial infection control and other quality improvement programs should be recognized. In US hospitals the quality assessment and improvement programs are commonly thought to include nosocomial infection control, risk management, monitoring of clinical outcomes, and care coordination. These programs are notable in using common data sources, applying common epidemiological methods, implementing similar analysis, sharing similar committee structures, and sharing similar goals of improved quality of patient care largely by avoiding increased nosocomial risk.[327] Recent emphasis on such issues as bioterrorism preparedness and patient safety, both closely related to infection control, highlights the need for the expertise of the healthcare epidemiologist in many arenas. The knowledge and skills of the healthcare epidemiologist also lend themselves extremely well to addressing many other issues at the forefront of patient care today. Knowledge of healthcare epidemiology is useful for drug use management, quality assessment, technology assessment, product evaluation, and risk management. In particular, application of healthcare epidemiology-based practices may offer much to the patient safety movement. These include establishing clear definitions of adverse events, standardizing methods for detecting and reporting events, creating appropriate rate adjustments for case mix differences, and instituting evidence-based intervention programs.[328,329]

10.3. Need and Efficacy in Developing Countries

With the exception of the WHO prevalence studies[4] and a few individual reports,[43,271] data on nosocomial infection risk in the developing countries are lacking. The potential for limiting loss of resources by preventing morbidity and mortality from nosocomial infections makes basic infection control appear even more critical in these countries, especially considering that the introduction of tools for sterilization, materials for disinfection and training in aseptic technique is relatively inexpensive. Indeed, while recent reports have noted the many challenges (e.g., administrative, financial) of establishing infection control programs in developing countries, the substantial impact of these programs has also been highlighted.[330]

10.4. Efficacy in Nonacute Care

Most studies of nosocomial infections have taken place in acute-care hospitals. A growing number of investigations are being reported in other healthcare settings such as nursing homes, rehabilitation, etc..[331,332,185,187–189] Nosocomial infection risk in other care settings, including ambulatory care, physician's office, and home care, is in the early stages of being studied and further characterized.[129,213,333]

10.5. Public Reporting of Hospital Infections

As noted earlier, mandatory reporting of healthcare-associated infection rates has become a reality. Although currently required in only a handful of states, mandatory reporting legislation is pending in over 20 additional states.[37] Although various professional organizations (e.g., APIC, SHEA, HICPAC) have all issued statements and published reports relative to the issue of mandated public reporting,[39,40] much uncertainty exists regarding the optimal approach. At this point, the specific healthcare-associated outcomes that are to be reported to the states are still being debated and differ across states. Furthermore, definitions of infections differ from state to state. In addition, there is currently no accepted method by which to risk adjust reported infection rates to account for differences in case mix across centers. Finally, the impact of public reporting on healthcare-acquired infections has not been evaluated.

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CHAPTER 27

Pertussis

Scott A. Halperin and Gaston De Serres

1. Introduction

Bordetella pertussis produces a single disease syndrome in man known as pertussis or whooping cough. The disease characteristically displays a protracted course measured in weeks to months, with the development of vigorous paroxysmal coughing often associated with vomiting and whoop. Complications include pneumonia; seizures; encephalopathy; hernias; rib fractures; and intracranial, retinal, and subconjunctival hemmorhage. At the turn of the century, it was a major cause of infant mortality worldwide. Largely due to immunization, the incidence of pertussis declined markedly during the middle of the 20th century; however, a resurgence of pertussis has been noted during the last several decades, even in countries with high immunization rates. The increased pertussis has mostly been observed in infants too young to have completed the primary immunization series and in adolescents and adults whose immunity has lapsed. In developing nations, pertussis continues to be a major child health problem.

2. Historical Background

The first recorded description of the disease dates from 1540 in England, and the first reported epidemic was in Paris in 1578.[1,2] Its absence from prior literature is curiously unexplained for a disease syndrome with such characteristic symptoms. Perhaps it attracted less attention than more devastating epidemic diseases such as plague and smallpox[1] or it may have been a disease of lower animals that became adapted to man.[3] This is supported by genomic analysis that found *B. pertussis* to be a derivative of *B. bronchiseptica*-like ancestors, a bacterium that affects animals.[4] It also has been suggested that smaller, isolated populations of the past with little contact among them could not sustain an organism with no carrier state or animal vector but productive of clinical immunity.[5] Identification and isolation of the responsible organism did not occur until the first part of the 20th century.[6] Because of poor understanding of the organism's biological anatomy as well as difficulties in propagation, attempts to develop effective vaccines were unsuccessful until the late 1930s. In the United States, whole, killed bacterial vaccines appeared to be of sufficient efficacy to warrant licensure and general use in young infants in the 1940s. Current federal regulations for vaccine production were established in 1953.[7]

Mortality from pertussis in the United States and other developed nations has declined throughout this century, even before the development and widespread use of pertussis vaccine and antimicrobial drugs.[8–10] Further decreases in death rates have occurred subsequently; the decline prior to the vaccine is not satisfactorily explained (see Section 5.1). But the fact that a decrease in mortality rates antedated widespread use of pertussis vaccine caused some authorities to question the necessity for the vaccine as a routine preventive measure for children,[11] particularly when there were allegations of severe adverse events against the whole-cell vaccines.

3. Methodology

3.1. Sources of Mortality Data

In the United States and other developed nations, the only sources of mortality information are published vital statistics. Case-fatality rates are inaccurate because the

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disease is underdiagnosed and underreported.[12–14] Using capture-recapture methodology, it was found that one- to two-thirds of all pertussis deaths were not accounted for in the national disease reporting system.[13,14] In addition, it is likely that an uncertain proportion of fatal cases are incorrectly diagnosed as other types of bronchopulmonary infections or sudden infant death syndrome.[15]

3.2. Sources of Morbidity Data

While intermittent active surveillance provides a good estimate of the incidence of pertussis, continuous determination of the precise incidence of pertussis in the United States and elsewhere is not presently possible for four reasons. First, the disease is often not reported. Second, the diagnosis may often be missed, even for cases with classical symptoms.[16] Third, it is clear that there are mild, unrecognized forms of the disease, especially in vaccinated individuals and older children and adults with waning immunity. Fourth, a syndrome that is clinically indistinguishable from true pertussis may be produced occasionally by two other *Bordetella* species: *B. parapertussis* and *B. bronchiseptica*. Certain adenoviruses or other viruses as well other bacteria may produce the syndrome.[17–21] Therefore, except during clear-cut, laboratory-confirmed outbreaks in defined populations, estimates of the incidence of pertussis are unreliable because nearly 90% or more cases are not diagnosed or reported.[12,16] Because mortality occurs mostly in infants and because of the inverse relationship of case-fatality with age, mortality does not reflect the true incidence in all age groups.[17] However, incidence and mortality rates in infants too young to be vaccinated ($<$ 2 months of age) are probably good indicators of the overall transmission in the population.[22]

3.3. Surveys

Surveys to determine proportions of the population immune to pertussis are of limited value for the reasons noted above, because of failure to recall the disease or prior immunization, and because of the lack of one serological test that directly correlates with clinical protection.[23] However, high serological titers of pertussis toxin (PT) correlate well with a recent infection.[24,25] PT titers remain high for a mean of 4.5 months, ranging from 4 weeks to 1 year, after disease onset. Seroepidemiological surveys have been conducted to document the incidence of infection that may or may not have caused disease.

3.4. Laboratory Diagnosis

Although laboratory tests are not required to make the diagnosis when an individual presents with all the classical symptoms of whooping cough, laboratory confirmation is still recommended. In young infants, and adolescents and adults, manifestations of pertussis may not be typical and laboratory confirmation is essential.[26] Although clinical diagnosis has a higher predictive value during outbreaks, laboratory confirmation is still beneficial, particularly if interventions such as chemoprophylaxis of close contacts are being considered. Many clinicians consider absolute lymphocytosis of 10,000 or more lymphocytes/mm^3 to be strong circumstantial evidence of pertussis in a child with appropriate symptoms. The magnitude of lymphocytosis roughly parallels the severity of the cough. However, lymphocytosis may be absent in very young infants. Additionally, disease-induced lymphocytosis may be reduced in previously immunized or infected persons because of the presence of antibodies to PT, which is responsible for the lymphocytic response.

The single, time-tested, unequivocal diagnostic test for pertussis is isolation of the organism on culture from a nasopharyngeal swab[12,27] or, preferably, nasopharyngeal secretion aspirate.[28] The usual medium for isolation of this fastidious, slow-growing organism is either modified Bordet–Gengou agar enriched with 15% defibrinated horse blood (needs to be prepared freshly) or Regan–Lowe medium containing 10% horse blood (8-week shelf life). Swabs should be inoculated immediately; delay of as little as 1 hour reduces the likelihood of recovery of the organisms because the organism is sensitive to drying. When inoculation must be delayed, Regan–Lowe transport medium (half-strength) or phosphate-buffered saline with 1% Casamino Acids may be employed. The addition of methicillin or cephalexin to the culture medium enhances the likelihood of recovery of *B. pertussis* by suppressing the growth of other organisms, but may inhibit growth of some *B. pertussis* strains. Cultures should be incubated at 35°C in moist air and examined daily for 7 days. Colonies are small and pearly and display a small zone of hemolysis on blood-containing media. Further identification of colonies is achieved by agglutination with commercially available sera or by direct immunofluorescence.

In experienced hands, identification by culture is nearly 100% specific; unfortunately, the sensitivity of culture methods is far less, even in experienced hands. Fresh media are required and, after the fourth week of illness, recovery of the organism is much less likely. Other factors that reduce the likelihood of recovering the organism on culture include prior administration of erythromycin or other

macrolides, past pertussis immunization, delays in transport of the specimen to the laboratory, and increasing age.[26,27,29] Additionally, many clinical bacteriology laboratories lack experience in the identification of *B. pertussis* on culture due to the relative infrequency of the disease.

Considerable efforts have been made to develop more sensitive and specific means for the identification of *B. pertussis* in respiratory secretions.[27] The most promising approach is the polymerase chain reaction (PCR), which has been employed in the diagnosis of other infections due to hard-to-recover organisms.[30–32] In brief, this technique identifies gene sequences unique to the organism in question. For detection, the method requires very few organisms in the specimen and therefore is highly sensitive. PCR is more sensitive than culture and as specific if care is taken to avoid cross-contamination of specimens and the environment.[33,34] Although universally accepted methodology for PCR does not yet exist, many clinical laboratories are now routinely using PCR in addition to or, more recently, in place of culture. Continued standardization of methodology is required to avoid "pseudo-outbreaks" resulting from false-positive PCR tests.[35,36]

Examination of direct smears from nasopharyngeal swabs by direct immunofluorescence (DFA) appears in some, but not all, studies to be approximately as sensitive as culture, but unfortunately, in most laboratories, lacks optimum specificity.[26,27] For this reason, direct immunofluorescence should not be used routinely for the laboratory diagnosis of pertussis and is no longer accepted as laboratory confirmation by some national surveillance systems.

Serological diagnosis of pertussis is becoming increasingly important, particularly with the increased number of cases in adolescents and adults. Use of specific antigens of *B. pertussis* in the serological assays has improved the diagnostic specificity of the test. However, like PCR, standardized clinical assays are not available. Both IgG and IgA antibodies are elicited after natural infection, whereas IgA antibodies are less commonly found after immunization. Antibodies to pertussis toxin are most specific for *B. pertussis* infections since there are no other cross-reacting antigens. Although demonstrating a rising antibody level in paired sera is the gold standard, this is often difficult because of the late collection of specimens, particularly in adolescents and adults who do not present for health care early in the clinical course of the illness. Single serum criteria for the diagnosis of pertussis using comparisons to population normal values show promise and have been used successfully for nearly a decade in Massachusetts.[37] Efforts continue to establish single serum antibody thresholds for the serological diagnosis of pertussis.[24,25]

4. Biological Characteristics of the Organism

B. pertussis is a small, poorly staining, gram-negative organism that appears in coccobacillary form on fresh isolation. Older cultures display pleomorphism, ranging from filamentous forms to larger bacilli. *B. pertussis* can be distinguished from *B. parapertussis* and *B. bronchiseptica* by various biochemical characteristics, agglutination, and immunofluorescence.

B. pertussis produces a wide array of biologically active materials that are associated with its virulence and are under the control of a Bvg operon. Table 1 lists some of the important somatic antigens, toxins, and biological activities of *B. pertussis*. From what is known of the biological activities of several of these antigens, it is possible to hypothesize about their roles in disease pathogenesis.[5,38,39] Of prime interest is pertussis toxin, a component of all acellular pertussis vaccines. PT appears to participate in the pathogenesis of pertussis at several stages by enhancing attachment of the organism to respiratory cilia, interfering with host defenses,

Table 1. Major Components of *B. pertussis*, Their Actions, and Their Putative Roles in Pertussis

Component	Physiologic actions	Pathogenetic role	Role in immunity
Pertussis toxin	Lymphocytosis promotion Histamine sensitization Insulin stimulation Mitogenicity	Attachment to cilia Cell toxicity	Major antigen
Filamentous hemagglutinin	None known	Probably facilitates attachment to cilia	Probably contributes
Agglutinogens	None known	None known	Possible
Adenylate cyclase	Compromises cell metabolism	Compromises bacterial killing	Possible
Endotoxin	Many	Not defined	Probably none
Tracheal cytotoxin	Toxic to respiratory epithelium	Possible cell toxicity	Probably none
Heat-labile toxin	Dermonecrotic and lethal in animals	Unknown	Probably none
Pertactin	None known	Unknown	Major antigen
Fimbriae	None known	Attachment to cilia	Possibly major antigen

and causing cell toxicity. Antibodies to PT can be measured serologically following immunization or natural disease, including mild or inapparent infections.[40] In animal models, PT causes lymphocytosis, islet cell activation and increased insulin secretion, and sensitization to histamine; its role in human lymphocytosis is likely.[26] PT also has been shown to inhibit chemotaxis of neutrophils and inhibit oxidative responses in neutrophils and macrophages.[41,42] PT is an ADP-ribosylating toxin secreted only by *B. pertussis* although *B. parapertussis* also possesses the gene encoding the toxin.[43] PT is a typical A–B toxin composed of six polypeptides. The enzymatically active A component is comprised of a single S1 subunit while the pentameric B oligomer is responsible for toxin binding and comprises S2, S3, two S4, and S5 subunits. The exact role of PT in human disease is unknown since, other than lymphocytosis and clinical disease severity, all of the symptoms of whooping cough can be caused by *B. parapertussis* which does not produce PT.[44]

Filamentous hemagglutinin (FHA) appears to participate with PT in facilitating attachment of the organism to respiratory cilia and is a component of most acellular pertussis vaccines. Whether it has other activities in relation to humans is unclear. FHA is a large filamentous protein regulated by the Bvg operon. The molecule has multiple binding domains including an Arg-Gly-Asp triplet (RGD domain) that is important for binding to monocytes/macrophages and other leukocytes. FHA also contains a carbohydrate recognition domain (CRD) responsible for its binding to ciliated respiratory epithelial cells and a heparin-binding site responsible for adherence to non-ciliated epithelial cell lines and hemagglutlination.[26] The role of FHA, as with PT, in human disease has yet to be established; however, measurable antibodies to FHA develop following immunization and the disease, including mild or asymptomatic infection.[5,40]

Pertactin is a 69 kilodalton membrane protein proposed to play a role in bacterial attachment.[45,46] Pertactin is also under regulation of the Bvg operon and contains an RGD binding site as well as other motifs that are commonly associated with eukaryotic cell binding.[47] Although it has not been possible to confirm definitively the role of pertactin in binding in animal models and in vitro, serological studies during the acellular pertussis vaccine studies suggest that antibody to pertactin is the most important antibody in predicting protection from infection.[48,49]

Fimbriae are filamentous protein cell surface structures present on many gram-negative organisms, typically involved in bacterial adherence. Fimbriae in *B. pertussis* are regulated by the Bvg operon and are likely involved in bacterial adherence to host epithelial cells and monocytes. A definitive role for *B. pertussis* fimbriae in adherence has been difficult to demonstrate because of the multiplicity of adhesins present in the bacteria. However, studies in animal models and in human vaccine suggest an important contribution of fimbriae to protection.[48,49]

Strains of *B. pertussis* can be serotyped and studied epidemiologically by heat-labile agglutinogens, antibodies to which can be measured serologically in humans. Among the three species of the genus *Bordetella*, 14 such agglutinogens have been identified; those designated 1 through 6 are unique to *B. pertussis*. Agglutinogens 1, 2, and 3 are those most commonly identified.[39] The identity of the agglutinogens has not been established although pertactin, fimbriae, and lipopolysaccharide all have agglutinogen activity. High levels of antibodies to agglutinogens (agglutinin antibodies) are associated with protection from infection after immunization with whole-cell pertussis vaccines in the original efficacy studies over 50 years ago.[50]

Adenylate cyclase is a component of the organism, largely somatic, that contributes to overcoming host defenses and, perhaps more importantly, facilitates cellular damage by inducing wasteful cell hypermetabolism.[38] Whether antibodies to adenylate cyclase, which is antigenic, contribute to clinical immunity is unknown.

Like other gram-negative bacteria, *B. pertussis* produces a lipopolysaccharide endotoxin that is mitogenic and pyrogenic and can induce tumor necrosis factor production by macrophages. The LPS of *B. pertussis* differs from those of *Enterobacteriaceae*, and its role in pathogenesis of the disease has not yet been elucidated.

There are several other components of *B. pertussis* that may well play roles in disease pathogenesis.[5,38,39] Among these are tracheal cytotoxin, which probably damages tracheal and bronchial epithelium, and a heat-labile toxin that is dermonecrotic or lethal and cytotoxic for bronchial epithelium in animal models.

5. Descriptive Epidemiology

The epidemiology of pertussis has been dramatically modified by immunization. However, even prior to widespread immunization in highly developed countries such as the United States and currently in less developed countries, pertussis has displayed distinctive epidemiological characteristics.

5.1. Prevalence and Incidence

Pertussis is a reportable disease. Because it is very contagious and is associated with mortality in infants and young

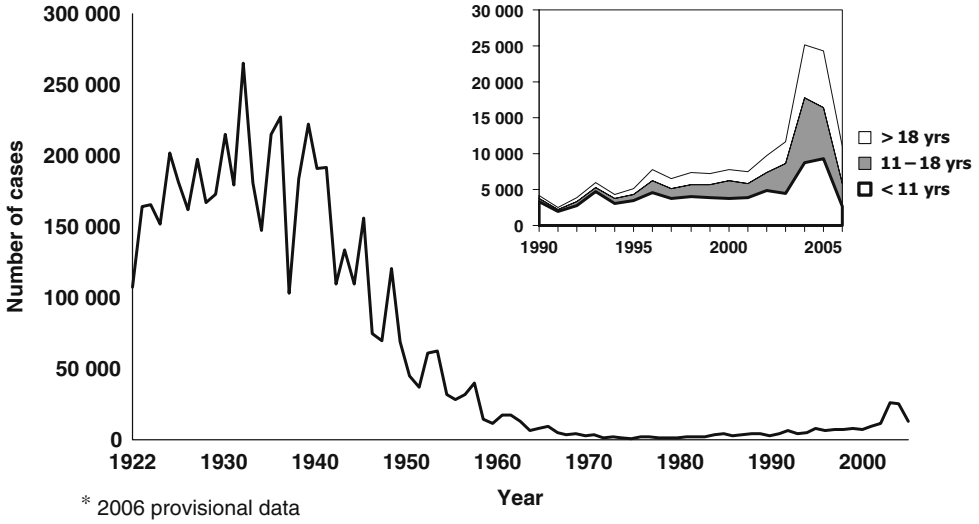


Figure 1. Reported number of pertussis cases in the United States, 1922–2006 (Source: Centers for Disease Control and Prevention (CDC) (75)).

children, it is important to make every effort to establish the diagnosis and report probable and proven cases to local health authorities. As noted above (Section 3.2), pertussis is vastly underreported, for the reasons that the diagnosis is often missed or is equivocal, medical care may not be sought, and some physicians may not be convinced of the importance of reporting. Since 1976 when pertussis notifications in the United States were at an historic low with 1,010 cases, reported cases have gradually increased to 25,827 in 2004, its highest count since 1959[51] (Figure 1). The incidence in 2004 was 8.9 per 100,000 and declined slightly the following 2 years.[52] After varicella, pertussis is currently the most frequently reported vaccine-preventable disease. This large increase is too great to be accounted for by population growth. How much this increase is an artifact attributable to augmented reporting because of better diagnostic procedures, enhanced awareness due to the alleged risks of pertussis vaccine being dramatically portrayed in public media, or other factors is unclear. This increase does not seem attributable to a decline in vaccine coverage. According to the National Immunization Survey (NIS), use of DTaP, DTP and DT was 66.3, 33.1, and 0.3%, respectively in children $\leq$18 months for 1998–1999.[53] For 2004, NIS reported that 95.9% of children 19–35 months of age had received $\geq$3 doses of DTaP, DTP, and DT.[54] While the pertussis increase affected all age groups,[55] it is remarkable in that the incidence in adolescents and adults has increased proportionately more than in young children.[51,56,57] As almost all adolescents and adults have been vaccinated during childhood, waning of vaccine-induced immunity is believed to be the main factor explaining their vulnerability.

While reported incidence in adults is still low, studies in adults with cough found that 12–32% had serologic, PCR, or culture evidence of pertussis infection.[58–69] Active surveillance for pertussis in coughing adolescents and adults found the incidence of laboratory-confirmed pertussis to range from 176 to 507 per 100,000 person-years.[64,70,71] Individuals with clinical disease represent only a part of all infected persons. Sera collected between 1991 and 1994 by the Third National Health and Nutrition Examination Survey (NHANES) were analyzed retrospectively and showed a rate of infection of 2,890 per 100,000 in individuals aged 10–49 years, with more frequent infections in adolescents and adults around 40 years of age.[25] A similar high rate of infection was also found in the United Kingdom.[72] A serological survey in Europe showed that rates of infection were highest in adolescents in countries with high vaccine coverage, whereas in countries with low coverage, infections were highest in children $<$10 years of age.[73]

Similar to incidence, reported mortality has also increased in the United States since the 1980s. There were 77 reported deaths between 1980 and 1989 and 91 between 1990 and 1999, for a mean annual number of 7.7 and 9.1, respectively.[74] This increase continued after 2000, and 27 deaths were reported in 2004.[75] Although it is clear that case-fatality rates from pertussis are inversely related to age, precise rates are difficult to determine, largely because of underreporting of the disease. Moreover, mortality rates in the developed world decreased in this century independent of immunization or any other form of specific intervention.[8,9,76,77] Direct evidence of the decline in mortality is limited to areas with optimum reporting of cases as

well as deaths, such as Providence, Rhode Island,[78] where case-fatality rates steadily declined from 1.36% in 1930–1934 to 0.24% in 1945–1949. Unfortunately, most information related to age-specific case-fatality rates is derived from children hospitalized with pertussis and thus is subject to the biases of such data. However, because almost everyone born between 1900 and 1949 experienced the disease during childhood, the decline in pertussis mortality in the developed world must be attributed to a decrease in case-fatality rates rather than reporting bias. For example, in the United States, annual deaths attributed to pertussis in children <1 year of age were 4.34 per 1,000 for the years 1900–1904; for 1935–1939, before specific measures such as immunization and antimicrobial therapy were developed, the annual death rate in this age group had declined 70% to 1.30 per 1,000.[9]

The reasons for this decline are unclear, but may be a consequence of a number of factors. These include better nutrition, the decline of debilitating conditions such as diarrheal diseases that compromised survival, quarantine of recognized cases, better supportive care, and decreasing birth rates that resulted in proportionately fewer infants being exposed to affected older siblings. In addition to those factors that predated the introduction of vaccination, mortality has also been significantly reduced by vaccination. The lowering of vaccine coverage pertussis in the United Kingdom during the 1970s was followed quickly by large outbreaks and numerous deaths.[79,80] When vaccine coverage increased again, the mortality plummeted.

There is also little doubt that advances in technologies available in intensive care units have resulted in the survival of the majority of infants critically ill with pertussis, many or most of whom would have otherwise succumbed. However, in less developed parts of the world, pertussis persists as a major cause of infant mortality. For 2002, the World Health Organization (WHO) estimated that 294,000 children died of this preventable disease prior to their fifth birthday.[81] While deaths in elderly individuals are rarely reported, pertussis caused an outbreak in a monastery where 53% of the 55–94-year-old residents had laboratory-confirmed pertussis and 10% of them died from intracranial bleeding while they were symptomatic for pertussis.[67] This outbreak was unusual in its severity but, with an increasing number of adult cases, it is possible that mortality will become an issue.

5.1.1. High-Risk Groups. Pertussis spreads easily in every group of the population but its incidence is highest in unvaccinated individuals who failed to be vaccinated, perhaps because they were too young or because they refused vaccination. The other group at high risk is broader and includes people in whom vaccine-induced immunity has waned.

For death, three risk factors are well identified: lack of vaccination, very young age, and prematurity. Nearly 90% of reported deaths occured in unvaccinated children.[74,82] Death occurs in the first months of life and is rare ($\leq$10%) after the first year of life. While 49% of the deaths occurred in children <2 months of age between 1980–1989, these children accounted for 66% of deaths in the 1990s.[74] Prematurity has also been associated with increased mortality. Among US children who died from pertussis in the 1990s, 51% were born at <37 weeks of gestation and 29% were born at <35 weeks. When limited to children with onset of pertussis between 2 and 11 months, the data show that 48% were born at <35 weeks.[74]

5.2. Epidemic Behavior and Contagiousness

Pertussis is both an endemic and an epidemic disease. In the absence of immunization, essentially no child escapes pertussis.[23] Indeed, during both World War I and World War II, pertussis, in contrast to other childhood contagious diseases, was rarely seen in military personnel, suggesting high prevalence of naturally acquired immunity.[1,78] Secondary attack rates in susceptible family members may reach 90% or more[23,83] and have been recorded as 100% in some studies.[17] There is, however, evidence that mild atypical cases occur in fully susceptible children.[78,84,85] Few data from the pre-vaccine era exist regarding transmission in school settings. However, one study examined an outbreak in an elementary school in Rochester, New York.[86] Excluding children with a past history of pertussis and those with exposure to a sibling with the disease at home, 40 (42%) of 95 school-exposed children developed the disease. Among presumably susceptible children, rates of whooping cough were higher in the lower grades, indicating that some of the older children had experienced forgotten or unrecognized infections in the past. While institutional[87–89] and local outbreaks continue to occur like in Cincinnati,[90] Chicago,[91] Massachusetts and Maryland,[37,92] Vermont,[93] Canada,[94,95] and elsewhere,[91,96] regional or statewide outbreaks have been more frequent than in the 1980s.[56,96]

The disease appears to be most contagious during the catarrhal stage (the first week of symptoms) and declines so that, as the paroxysmal cough begins to wane, infectivity generally disappears. Early studies in unvaccinated children found that a majority (62–83%) with pertussis had a positive culture up to 21 days after onset of symptoms.[97,98] During the fourth and fifth week of disease, the proportions decreased to 32–39% and 11–15%, respectively . Other more recent studies including mostly unvaccinated children confirmed that culture remains positive in about one-third of

cases during the fourth week and in more than 10% during the fifth week.[99,100] The proportions were not very different in vaccinated cases.[100] However, contagiousness of vaccinated cases is reduced as compared to unvaccinated ones.[101] So even if contagiousness is much lower 3 weeks after the onset of disease, a percentage of cases can still transmit after this period.

While it is still uncertain whether pertussis can be transmitted by individuals without overt disease, asymptomatic vaccinated individuals can be culture-positive for pertussis but the number of bacteria is much lower than in symptomatic cases.[102] These individuals, as well as those immunized in young childhood who experience a mild, clinically unrecognizable disease in adolescence or young adulthood, may serve as a source of exposure. Currently there is no evidence of a long-term asymptomatic carrier state.

5.3. Geographic Distribution

The distribution of pertussis is worldwide, though clearly modified by immunization and other poorly defined social, economic, and nutritional factors. The number of cases of pertussis occurring worldwide cannot be estimated with accuracy. Reported incidence rates vary among different nations; the low incidence in some countries may represent poor case reporting, herd immunity from preceding outbreaks, or high levels of immunization. The highest rates occur in those developing countries where immunization programs are inadequate. However, as measured by death rates from pertussis, the incidence must be declining as a consequence of the Expanded Programme on Immunization.[81,103]

5.4. Temporal Distribution

Pertussis outbreaks occur at any time but are perhaps more frequent in the summer. Pertussis is reported more often in summer in younger children.[96] In the past this was believed to be due to increased contacts between young children during warmer months. In persons aged 5–19 years, the peak of the season is between October and December[56] or even during winter months.[94] Different seasonal patterns have also been reported from various countries.[104]

Outbreaks occur every 3–5 years; in isolated areas, intervals of relative freedom from pertussis sometimes extend beyond 6 years.[78,105] While the incidence of disease has decreased with immunization programs, the interepidemic period has not increased, suggesting that the rate of infection in the population has not been reduced substantially.[23]

5.5. Age

Since the introduction of vaccination, there has been a noticeable shift in the age distribution of reported cases of pertussis. For the years 1935–1939, prior to the availability of pertussis vaccine, data from ten states indicated that, for whites, about half of all cases of pertussis were reported in 5–14-year-old children;[8] for blacks, this proportion was lower (20–40%), with the preponderance of cases occurring before 5 years of age. Less than 15% of cases in whites occurred before 1 year of age, but the proportion of cases in infancy among blacks was nearly twice that in whites, probably for socioeconomic reasons.[8] The proportion of reported cases in persons 15 years and older was $<2\%$ in the late 1930s.[8]

Widespread use of pertussis vaccine during the past five decades has been associated with a marked decline in reported cases and with a striking shift in the proportions of cases in various age groups. For 1980–1989, 50% of cases were reported in infants in contrast to 12.5% in 2004[51,96] whereas these proportions in children 5–14 years old were 17 and 30%, respectively. The greatest change, though, was in persons 15 years and older where the number of cases increased from 12 to 41% during the same period.

Infants too young to have been vaccinated have the highest incidence of pertussis (Figure 2). While the incidence in infants 5 months and older remained fairly stable, it increased substantially in younger infants since the mid-1990s. During the same period, incidence has also increased in older children and adults, with the incidence of pertussis in children and adolescents 10–19 years of age now surpassing that of younger children (Figure 3). The cause of this generalized phenomenon is unclear but may be an artifact of better diagnosis and enhanced reporting or waning immunity (see Section 7.2).

5.6. Sex

Although both sexes are presumably equally affected, pertussis is unusual in that, inexplicably, the disease is more often reported in females. It may be that the smaller larynx of female infants places them in greater jeopardy of severe disease. While females formerly exhibited higher mortality rates, in the 1990s, deaths were equally divided between sexes.[74]

5.7. Race/Ethnicity

Death rates were formerly higher in blacks[8] but Hispanics have been the most affected group in recent years;[74] it is

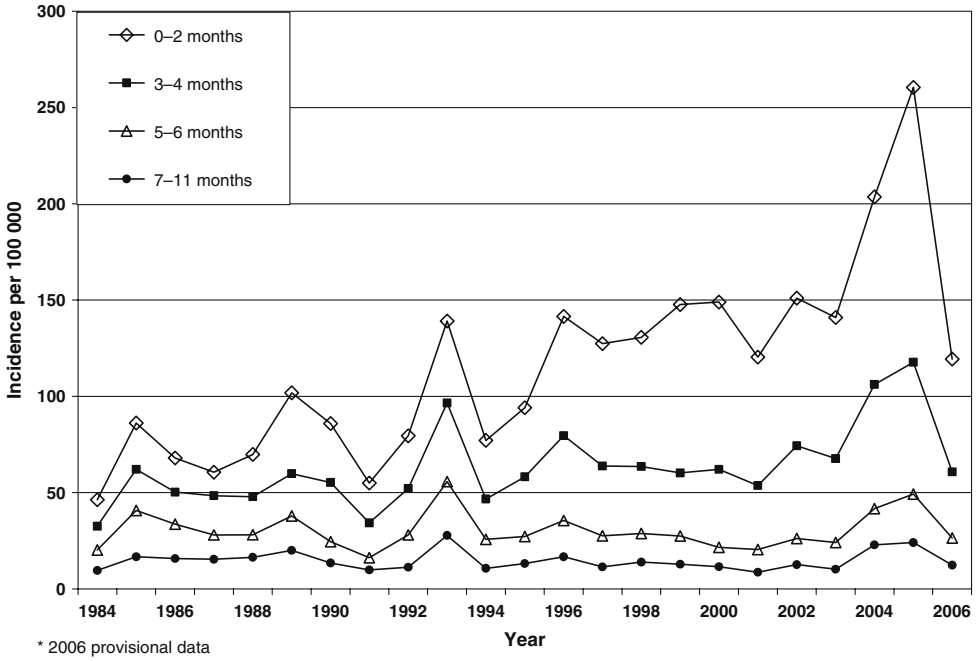


Figure 2. Reported incidence per 100,000 of pertussis among infants in the United States, by month of age, 1984–2006 (Source: CDC (75)).

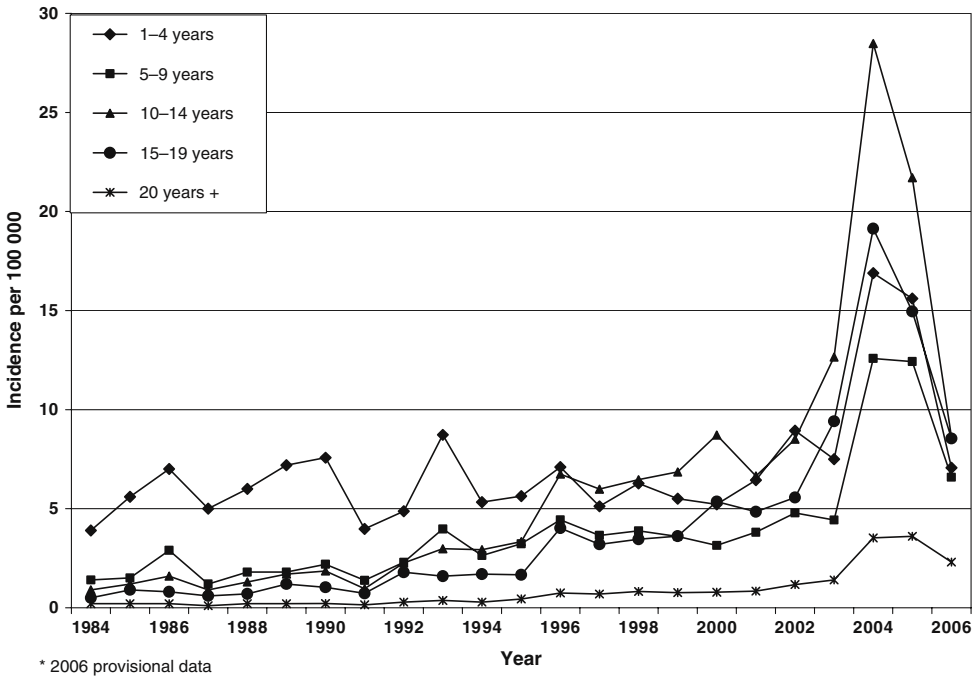


Figure 3. Reported incidence per 100,000 of pertussis by age groups in the United States, 1984–2006 (Source: CDC (75)).

likely that these higher rates are attributable to lower vaccine coverage and socioeconomic and other factors, rather than to race.

5.8. Other Factors

Mortality rates are higher in lower socioeconomic groups, probably because of exposure earlier in life due to crowding and close contact. However, case-fatality rates are also inversely related to socioeconomic status for reasons that are unclear;[19] access to health care and nutrition may play roles. In contrast to many infectious diseases, mortality from pertussis has been higher in rural than in urban areas.[78]

6. Mechanisms and Routes of Transmission

Pertussis is transmitted via close contact by droplets. It is highly contagious; indeed, up to 90% of susceptible family contacts will develop clinical disease from an index case in the household.[2,83,106–109] There is no evidence of spread by other routes such as airborne droplet nuclei, fomites, or dust.

7. Pathogenesis and Immunity

7.1. Pathogenesis

The pathogenesis of the disease, better defined in recent years, comprises attachment of the organism to tracheal and bronchial cilia, evasion of host defense, induction of local tissue damage, and production of systemic manifestations. Cellular invasion occurs but systemic dissemination does not. Adherence is mediated by multiple adhesins, including pertussis toxin, filamentous hemagglutinin, pertactin, fimbriae, and others (lipopolysaccharide, BrkA). The redundancy of the adhesins has made it difficult to determine which is of primary importance. Evasion of host defenses is achieved by pertussis toxoid and adenylate cyclase toxin which inhibits migration of lymphocytes and macrophages and interferes with neutrophil phagocytosis, respectively. Local tissue damage results from tracheal cytotoxin, dermonecrotic toxin, and lipopolysaccharide.[26] The cough appears to be due to local tissue damage and interference with normal mechanisms of bronchial toilet; mucoid secretions accumulate and are responsible for ineffective, repetitive, paroxysmal coughing and, ultimately, for varying degrees of bronchial obstruction, atelectasis, and bronchopneumonia.[2] However, the duration of symptoms exceeds the duration of the local damage so other unknown toxins or mechanisms may contribute to the clinical manifestations. Bronchopneumonia is the most frequent serious complication of pertussis. In older children, it most often results from invasion by nonspecific respiratory flora or other respiratory pathogens such as *Streptococcus pneumoniae*. In very young infants, invasion by *B. pertussis* itself with a primary pertussis pneumonia is common.[82] Pneumonia occurs in up to 10% of infants and the elderly with pertussis and less frequently among older children, adolescents, and young adults.[110–112] Other systemic manifestations such as lymphocytosis and hyperinsulinism resulting in hypoglycemia are caused by the actions of pertussis toxin.

A major complication of pertussis is encephalopathy, varying in manifestations from short-lived convulsions to intractable seizures, coma, and permanent cerebral damage.[9,17] The mechanism of pertussis encephalopathy is unclear. The assumption that encephalopathy results from the direct effects of one or more of the toxins of *B. pertussis* no longer appears tenable, and instead it is likely that anoxia and, in some instances, cerebral hemorrhages are responsible.

The experience of contagious disease hospitals and institutions for the handicapped in the past indicate that pertussis encephalopathy is far from rare,[17,113,114] but few data are available. One estimate may be derived from cases reported from the contagious disease hospital that served residents of Brooklyn, New York, in the 1930s.[115] The childhood population of Brooklyn can be approximated from birth records and, assuming that every child in Brooklyn experienced pertussis, the incidence of severe encephalopathy was approximately 1 in 22,000 cases. A more precise estimate, made by the British National Childhood Encephalopathy Study (NCES), approximated a frequency of 1 in 11,000 cases.[116] More recently, in hospitalized cases of pertussis, seizures have been reported in 0.8–2.0% and encephalopathy in 0.1–0.4% of cases.[53,111]

7.2. Immunity

The duration of immunity after clinical pertussis is not known; although previously thought to be lifelong, this is now known not to be the case. In recent years, several well-documented series of pertussis in adults have been reported[117–123] including instances of recurrent, laboratory-confirmed cases. In many instances, these illnesses are mild and atypical but classical pertussis with complications can occur.[124,125] It appears that pertussis in adults is more common than formerly appreciated and may occur in persons who experienced the disease in the past as well as in those who were actively immunized as children.[26] The increased rates of disease in adults may be the result of both better recognition and an actual increase in rates in older persons.

If there is a true increase, it is tempting to hypothesize that immunity, whether induced by pertussis or by the vaccine, has been more apt to wane in recent decades due to the present infrequency of exposure to the infection and the consequent lack of repeated, casual "streetcar" boosters.[119] Whatever the reason, it is apparent that pertussis in adolescents and young adults is a problem of some importance, particularly because such persons may constitute a reservoir of infection and a source of transmission to others, including young infants.[117,119,120,122]

The mechanisms of clinical immunity to pertussis have escaped definition for many years, and only in the last two decades has much progress been made. This progress has resulted from laboratory studies that have defined much better the components of the organism and from new methods of measuring antibodies serologically. Several antigens have been identified that appear to be important in the pathogenesis of pertussis and in addition induce antibodies, one or more of which may provide clinical protection against the illness (see Section 4). One of these is PT, which appears to be the major antigen that produces immunity in the mouse protection test, long used for laboratory evaluation and standardization of pertussis vaccine. Pertactin has also been identified as an important antigen that best correlates with protection after immunization.[48,49] FHA also may be important not only in disease pathogenesis but also for induction of clinical immunity. FHA is thought to facilitate attachment of *B. pertussis* to respiratory cilia; however, in the acellular pertussis vaccine efficacy trials, FHA did not appear to contribute to increased protection.[48,49] Fimbriae have also been shown to be important in immunity after immunization, particularly in the protection against mild illness.[126] The agglutinogens may relate to the induction of clinical immunity as well; three of them appear to be of consequence. Their relation to disease pathogenesis is uncertain, but general studies in the past have suggested correlation between clinical immunity and levels of antibody against agglutinogens.[83] Further, there is some indication that a mismatch between the specific agglutinogens in pertussis vaccine and those of circulating *B. pertussis* strains is associated with diminished vaccine efficacy.[127]

There is a probable role for secretory IgA-mediated immunity;[128] IgA antibodies against specific pertussis antigens can be detected in the saliva and nasopharyngeal secretions after pertussis infection, although not usually after immunization. In addition to humoral immune responses, cell-mediated immune responses to pertussis antigens have been measured after human infection and in animal models. A Th1 response to PT, FHA, and pertactin occurs after natural infection and after immunization with the whole-cell vaccine. A Th2 response occurs primarily after immunization with acellular pertussis vaccines.[129,130] The relative contributions of humoral and cell-mediated immunity in protection against infection and recovery from infection has yet to be elucidated.[131]

8. Patterns of Host Response

8.1. Clinical Features

The incubation period of clinical pertussis is usually 7–10 days and rarely longer than 2 weeks. The first week or two of the illness is called the catarrhal stage, because initial symptoms of the disease are nonspecific, including mild but gradually increasing cough, nasal symptoms suggestive of a cold, slight fever, and some anorexia.

As the catarrhal stage progresses, the cough increases so that severe spells of coughing with the characteristic whoop appear about 2 weeks after onset, marking the beginning of the paroxysmal stage. The paroxysmal cough results from an inability to expel tenacious mucus; the whoop is created by vigorous inspiration through the glottis at the end of the paroxysm. Paroxysms may occur repeatedly and may be initiated by eating, drinking, talking, crying, examination of the throat, or even by hearing another individual cough (such as occurs in a hospital where there are other children with pertussis). During paroxysms, cyanosis may occur. Repetitive post-paroxysmal vomiting may be sufficiently prolonged to cause substantial weight loss. Fever is usually low and may be throughout the course of pertussis in the absence of secondary infection. Subconjunctival and cerebral hemorrhages and epistaxis, umbilical and inguinal hernias, and rib fractures may be seen. Convulsions, with or without encephalopathy, and pulmonary complications occur during the paroxysmal stage.

In classic pertussis, the duration of the paroxysmal whooping stage is 4 or more weeks, with increasingly frequent paroxysms for 1–2 weeks and gradual subsidence and ultimate disappearance of the whoop thereafter as the patient enters the convalescent stage. Cough may persist for some weeks into the convalescent stage, and exacerbations may occur for up to 1 year if an intercurrent respiratory infection occurs. Mortality from pertussis is inversely related to age. The case-fatality rate in hospitalized infants is approximately 1%; after 5 years of age, it is negligible.

The major causes of mortality appear to be pulmonary complications including overwhelming pertussis pneumonia and pulmonary hypertension, with some contribution from encephalopathy and other neurological complications.[82] Secondary or intercurrent affections, such as otitis media,

mastoiditis, inanition, and diarrhea, undoubtedly contributed to mortality in the past in the United States and continue to do so in developing countries.

Permanent neurological sequelae from pertussis, especially from pertussis in infancy, undoubtedly occur.[114–116] More subtle intellectual or behavioral abnormalities also may result in some instances.[113,132] Given the high mortality after severe pulmonary infection and the failure of intensive therapies such as extracorporeal membrane oxygenation to alter the outcome,[82] long-term pulmonary damage has not been described.[133,134]

8.2. Diagnosis

The diagnosis of the full-blown pertussis syndrome is readily made on clinical grounds by most clinicians who have had experience with the disease. The protracted course, paroxysmal coughing, and whoop are unmistakable. A history of exposure or knowledge of other cases in the area is of help.

Immediate support for the diagnosis of pertussis frequently may be obtained from the leukocyte count. Absolute lymphocytosis, often striking, parallels the severity of the cough, but is said to be less characteristic in young infants or adolescents and adults.[19] A definitive diagnosis may be established in two ways. First, recovery and identification of *B. pertussis* by culture or PCR of respiratory secretions is definitive (see Section 3.4). However, isolation on culture is not accomplished easily and care must be taken to avoid cross-contamination of PCR specimens during collection and specimen processing. Identification of the organism in respiratory secretions by fluorescent-antibody techniques is compromised by frequent false-positive tests[12,27] and is less sensitive. Serological testing for the diagnosis of pertussis has been improved considerably by testing paired acute and convalescent sera by enzyme-linked immunosorbent assays (ELISA).[12,27,120] Using this technique, antibody responses to PT, FHA, pertactin, and fimbriae of *B. pertussis* can be measured, and the method is highly sensitive and specific.[12,27,37,40,120,128] Although IgM responses to infection (and immunization) occur, the rise and fall of these responses is usually too rapid to be of diagnostic utility. Potential limitations of the ELISA method include failure to recognize an IgG response in a previously immunized individual because the rapidity of anamnestic responses may result in a rise in IgG antibodies before the acute serum is obtained. In such instances, infection may be recognized by a primary IgA response because IgA antibodies are not induced by immunization.[128] More recently, serum IgA responses against several antigens have been demonsrated after acellular pertussis vaccine. It also has been shown that single convalescent ELISA serum antibody levels that exceed the mean of a control population by three standard deviations are of diagnostic value when timely acute serum samples were not obtained.[37,40,120,121] Additional studies suggest that exceeding a population-derived level of anti-PT antibody can be used to diagnose pertussis as long as it has been at least 3 years since a previous dose of acellular pertussis vaccine.[24,25] Treatment of pertussis with erythromycin or other macrolides appears to have little or no effect on antibody responses.[135]

Because no absolute serological correlate of protection has been identified for pertussis, a standardized case definition was established to measure the efficacy of candidate acellular pertussis vaccines. The most widely accepted case definition established through the World Health Organization defined typical pertussis as 21 or more days of paroxysmal cough plus one or more of the following: a positive culture; a significant rise in IgA or IgG antibodies to FHA, PT, or fimbriae 2 and 3; or household exposure to a culture-positive case within 20 days before or after disease onset in the subject in question.[136] This definition is associated with high specificity but lower sensitivity. For surveillance purposes where higher sensitivity is desired, even at the expense of specificity, the Centers for Disease Control and Prevention (CDC) has defined pertussis clinically as a cough illness lasting at least 2 weeks with one of the following: paroxysms of coughing, inspiratory "whoop," or post-tussive vomiting, without other apparent cause (as reported by a health professional).[137]

9. Control and Prevention

In the absence of widespread immunization, outbreaks of pertussis cannot be prevented and control is difficult. Rates of transmission to susceptible households may be 90% or higher; in classroom situations, up to 50% of susceptible students in the same room may acquire the disease following exposure to an infected child (see Section 5.2).

9.1. Isolation

Isolation or quarantine of children with clinical whooping cough is of limited value in controlling spread of the disease, for the reason that children in the catarrhal stage, before the characteristic whoop appears, are quite infectious.

To prevent transmission, recognized cases should be excluded from school and contact with other children for 5 days after beginning effective antimicrobial treatment. The

carrier state in pertussis has not been demonstrated; if such exists, it is doubtful that it plays an important role in transmission because of the lack of associated cough. Milder forms of the disease, which occur in partially immune individuals, probably play a role in transmission (see Section 6).

9.2. Antibiotics

Pertussis is relatively insusceptible to antimicrobial therapy clinically. Although the organism is susceptible in vitro to various antimicrobial drugs effective against gram-negative organisms, in vitro susceptibilities do not correlate with clinical effectiveness. Macrolide antibiotics including erythromycin, clarithromycin, and azithromycin are effective in eradicating the organism from the nasopharynx within 5 days and may therefore reduce transmission. Duration of therapy with erythromycin has generally been recommended for 14 days, although 7 days has been shown to be equivalent;[138] 5 days of azithromycin was equally effective.[139] Antibiotic therapy does not have a substantial effect on the clinical course of the disease unless given early during the catarrhal phase.[135,138–143] By the time the patient is well into the catarrhal stage or has begun to whoop, benefit is not expected. Because exposure is often unrecognized and because the disease is rarely suspected during the catarrhal stage, antibiotics do not comprise a satisfactory approach to disease control.

Macrolides administered as chemoprophylaxis to patients' contacts have been used for decades, but there is great controversy over the efficacy of this intervention.[142] While epidemiological studies found some benefit,[143–145] randomized clinical trials did not.[140] Administration of chemoprophylaxis very early after exposure is probably key to obtain some results, but this is unfortunately rarely possible given the delay between onset of disease and the diagnosis of pertussis in the index case. Given the uncertainty about its efficacy, Canada and the United Kingdom restricted chemoprophylaxis mostly to situations involving risks of pertussis in an infant. In the United States, chemoprophylaxis is used for broader indications. There is clearly a need for better evidence regarding the efficacy of chemoprophylaxis.

9.3. Passive Immunization

Passive protection of exposed, susceptible individuals was attempted in the past with varying results. In the late 1930s, convalescent and hyperimmune sera were administered to susceptible children exposed within the same household. In one small controlled study, protection appeared to be as high as 70%.[106] Subsequently, pertussis IgG, prepared from human or rabbit sera, was employed with negative results.[148] More recently, reports from Sweden and Japan have indicated that human hyperimmune globulin containing anti-FHA and anti-PT effectively ameliorated the course of pertussis,[147, 148] and a study in the US suggested benefit with an anti-PT hyperimmune intravenous immunoglobulin.[149] However, an efficacy study with the product was terminated early because the product was no longer available; no benefit was apparent at the time the study was ended.[150]

9.4. Active Immunization

Prior to World War I, ill-controlled, hit-or-miss attempts were made to develop an effective pertussis vaccine, with inconclusive or uninterpretable results. By the mid-1940s, whole-cell killed pertussis vaccine was widely used in the United States. Current criteria for standardization of the vaccine in the United States were established in 1953 by federal regulation.

There is little question that most properly immunized children are protected against pertussis on exposure.[39,151] However, the proportion of children actually protected when intimately exposed, such as in the home, is less than 100%.[152] The severity of the disease in previously immunized children is usually milder. Estimates of vaccine efficacy in studies of exposed immunized and unimmunized children vary remarkably because of differences in study methods and in ascertainment, particularly in relation to recognition of infection versus clinical disease, as well as differences between various whole-cell vaccines.

Concerns about the safety of whole-cell vaccines led to the development of acellular pertussis vaccines. Three general types of reactions have been attributed to whole-cell pertussis vaccines. The first of these comprises mild local reactions at the site of injection and slight systemic reactions, such as fever and malaise, as observed following receipt of most vaccines. These are usually of no concern. A second group of reactions includes two that are quite disturbing but appear to be without permanent sequelae. These include incessant, inconsolable crying and a strange, shock-like syndrome following injection in young infants. The crying is very likely due to pain at the site of injection. The cause of the shock-like hypotonic-hyporesponsive episodes has not been identified but is also likely due to a pain response. Though these episodes are frightening, death or other sequelae in association with them has not been described. The third group of events includes those related to the central nervous system. These events range from what appear clearly to be simple febrile convulsions without sequelae[153] to severe encephalopathy with permanent brain damage or

death. Although it is clear that pertussis vaccine is the antigen in DTP (pertussis vaccine combined with diphtheria and tetanus toxoids adsorbed) that contributes most to fever and local reactions, it has not been shown that it is the component primarily responsible for the other phenomena.

A 1981 study provided somewhat more precise information about the frequency of the first two groups of side effects.[154] In this study, infants and children who received a total of 15,752 inoculations of DTP and 784 of DT (diphtheria and tetanus toxoids absorbed) were followed for 48 hours. Local and systemic reactions, including fever, were considerably more frequent following DTP than DT. Nine children experienced short seizures following DTP; all but one episode were associated with fever. Nine other children had hypotonic–hyporesponsive episodes. Thus, each of these reactions occurred at a rate of 1/1,750 injections (95% confidence interval: 1/925 to 1/3,850). However, attribution of these two more worrisome types of reactions solely to the pertussis component of DTP was not feasible because too few immunizations (784) with DT were monitored to permit comparison.

Estimates of rates of post-pertussis-vaccine encephalopathy have ranged from 1/50,000 to 1/300,000 or more;[155] subsequently, a well-designed study that controlled for background rates of encephalopathy suggested that serious neurological reactions occur following 1/110,000 injections and that about one-third of children so affected incur sequelae that may be evident 10 years later.[116,156] However, there are doubts about the precision of this rate, and indeed it has been concluded by the investigators that the results show that pertussis vaccine produces permanent brain damage very rarely or not at all.[156] Instead, it may be that the majority, if not all, of the instances of alleged pertussis vaccine encephalopathy represent either coincidence or the precipitation of inevitable manifestations of pre-existing disorders by the well-known systemic effects of DTP. Most authorities have concluded that permanent brain damage as a result of DTP is either a myth or is so rare that it cannot be differentiated from coincidence. Although occasional reports have suggested that DTP may precipitate the sudden infant death syndrome on rare occasions, several have indicated that these events represent simple coincidence.[157] A full review of all reports and studies has absolved the vaccine of most, if not all, of the alleged high incidence of serious or fatal reactions.[157] This and other assessments have resulted in the lessening of public concerns about risks attributable to the vaccine.[158]

Doubts about the continuing need for pertussis vaccine plus anxiety about its safety, widely publicized in the media, resulted in striking diminutions in pertussis vaccine use in the late 1970s in Britain and Japan because of the concerns not only of parents but also of physicians. In both countries, widespread outbreaks of pertussis with its attendant mortality, particularly in unimmunized infants, ensued.[152,159] In Sweden, pertussis immunization was discontinued in 1979 because of doubts about vaccine efficacy, and similarly a recrudescence of the disease occurred.[160] These natural or inadvertent experiments provide strong evidence of the public health merit of continued widespread immunization against pertussis. Any risk of serious disability or death from pertussis vaccine is negligible or nonexistent, and therefore the benefits far outweigh this possibility.

In spite of this, it was clear that it would be advantageous to have an improved pertussis vaccine. First, the whole-cell vaccine undoubtedly contains antigens and other substances that not only are reactive (though not truly dangerous) but also are irrelevant to immunity and contribute to physicians' and parents' concerns about DTP. Second, from the standpoint of children, a less unpleasantly reactive vaccine is desirable and would be more acceptable in both developed and developing countries. Third, booster doses for adolescents and adults are needed to control pertussis and whole-cell pertussis vaccines are poorly tolerated in older children and adults. For these reasons and the fact that a much better understanding of the biological anatomy of *B. pertussis* has evolved steadily, acellular vaccines have been developed and are now used exclusively in most developed nations.

It is clear that these acellular vaccines are at least as immunogenic and far less reactive than whole-cell DTP, including in infants.[39,161,162] A number of randomized, controlled clinical trials of various acellular DTP products (DTaP) were conducted in infants. Efficacy was compared with whole-cell DTP. The results of these studies showed clinical protection comparable to the whole-cell product.[163]In studies in Sweden[84] and Italy[85] supported by the US National Institutes of Health, two acellular pertussis vaccines comprising PT, FHA, and pertactin and a third comprising PT, FHA, pertactin, and fimbriae 2 and 3 were 85% effective against the WHO case definition of pertussis. In other studies, vaccines containing PT alone,[164] PT and FHA,[165,166] and PT, FHA, pertactin, and fimbriae[167]were also effective. Although the methodology of the studies varied, thereby making direct comparison of all of the vaccines impossible, most experts have concluded that acelluar pertussis vaccines containing at least PT, FHA, and pertactin were superior to PT or PT and FHA vaccines and that fimbriae may provide benefit against milder cases of pertussis.[126,163] Long-term follow up of these studies has demonstrated persistence of protection out to six years; surveillance for continued protection is ongoing.[168]

From these studies, it may be concluded that acellular pertussis vaccines appear to be as protective as whole-cell DTP in infancy. The improved safety profile of the

acellular pertussis vaccines has also led to less reports of encephalopathy, hypotonic-hyporesponsive episodes, and febrile seizures.[169,170] Injection-site reactions are initially less common with the use of acellular pertussis vaccines but increase after the fourth and fifth consecutive dose of an acellular product.[171–173] However, injection-site reactions associated with the acellular pertussis vaccine are characterized by painless redness and swelling compared to painful swelling associated with the whole-cell vaccine. Thus, these injection-site reactions appear to be of far less concern to the children and their parents.[174]

In view of the rising incidence of pertussis amongst adolescents and adults and the concern that these individuals serve as the reservoir for infection in young infants, acellular vaccines formulated for adolescents and adults have been developed and are now licensed through much of the world. In North America, acellular pertussis vaccines are recommended at 2, 4, 6, and 18 months of age with a booster prior to school and in early- to mid-adolescence.[175,176] A single dose of an acellular pertussis-containing vaccine is also recommended for all adults;[177] the need for repeat doses in adults awaits data on antibody persistence.

10. Unresolved Problems

Despite the use of effective vaccines in children, in the past decade, reported pertussis incidence has been increasing in many countries with high vaccine coverage such as Canada, Australia, and the United States. This increase, due to waning of vaccine-induced immunity, is occurring mostly in adolescents and adults in whom pertussis is better recognized and probably more frequent. With the implementation of adolescent vaccination programs, there will remain two areas for better control: adults and newborns.

As acellular pertussis vaccines administered to adolescents will not provide a lifelong protection, repeated booster doses will be needed to protect adults. It will be important to determine the interval between booster doses required to maintain protective immunity. The duration of this interval will be critical in assessing the cost and feasibility of programs aimed at preventing pertussis throughout the entire lifetime. Better information is also needed to determine the burden of pertussis illness and the immunogenicity of the acellular pertussis vaccine in the elderly in whom the disease is more severe. In parallel, it will also be important to estimate more accurately the duration of the protection induced by natural (re)infection. These (re)infections, symptomatic or not, are frequent in adults, play a central role in the current epidemiology of pertussis, and may be modified by adolescent vaccination programs.

Development of effective interventions to prevent pertussis in newborns is the other great challenge. Maternal immunization during pregnancy and vaccination of household contacts of the newborns have been proposed but proof of their efficacy is lacking.

Further improvement in diagnostic tests for pertussis is also a research priority in view of the poorer performance of the current tests in adolescents and adults. Standardized, single-serum diagnostic tests and point-of-care testing would greatly facilitate both the diagnosis of disease and the accurate determination of the burden of disease in various subpopulations.

Further research is needed to determine the efficacy of chemoprophylaxis of household contacts to prevent secondary cases. Finally, while antibiotics in cases do reduce shedding and transmission, they do not have a significant impact on symptoms. There is a need to develop a treatment that will stop or alleviate the symptoms of pertussis.

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CHAPTER 28

Plague

David T. Dennis and J. Erin Staples

1. Introduction

Plague is a zoonotic infection of rodents caused by *Yersinia pestis*. Fleas transmit the infection between rodents and incidentally to humans and other susceptible mammalian hosts. Inoculation of the plague bacillus through the skin or mucous membranes produces an acute illness with fever and swollen, painful, regional lymph nodes, the hallmark of *bubonic plague*. Bloodstream invasion may lead to *septicemic plague* or to establishment of infection of other organ systems such as the lung or the meninges. Systemic spread to the lungs may result in *secondary pneumonic plague*, which can then be transmitted by the airborne route via infective respiratory droplets. Airborne transmission is uncommon and typically results at most in one or a few cases of *primary pneumonic plague* among close contacts of the coughing patient; rarely, an outbreak may occur.

Historical epidemics of plague spread rapidly. Attack rates and fatality ratios were high: 50–60% of bubonic patients died; pneumonic and septicemic patients almost invariably experienced a fulminant, fatal course. Prevention measures seemed futile, and epidemics understandably caused panic and upheaval. Even today, despite the availability of curative antibiotics and effective control measures, plague provokes alarm and irrational responses. The increasing concern with bioterrorism and the designation of *Y. pestis* as a Category A select agent have led to renewed concerns about the disease.[1,2]

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2. Historical Background

The earliest description of pandemic plague is that of the Great Plague of Justinian in the 6th century AD, which appeared first in Egypt and extended through the Middle East, North Africa, and parts of Europe. According to some accounts, half the population of the Roman Empire died from this scourge. However, descriptions of clinical illness and measures of mortality in this and other historical epidemics are poorly documented.[3,4]

In the 14th century, pandemic plague recurred. This medieval pandemic is thought to have begun in central Asia, first striking China and Northern India before moving along trade routes to the Crimea and the Levant. From these points, it spread by ship to Italy in 1347, and onward through Europe and to England in 1348. Records indicate fatality rates as high as 40% or more in some concentrated European population centers, and the epithet "The Great Mortality" was applied with good reason;[3–5] "Black Death" was coined later, specifically in relation to epidemics that had great numbers of pneumonic plague.[6] The practice of quarantine, a waiting period before docking of ships arriving from plague-affected areas, was initiated by Italian authorities during this pandemic.

Plague intermittently re-emerged in Europe during each of the four succeeding centuries. The "Great Plague" of London peaked in 1665 when deaths in August and September of that year were reported to be 7,000 per week in a population of 500,000. Plague gradually disappeared from the region in the 18th century, and Europe was apparently free of plague by 1840.[5] Zinsser[7] considered that this disappearance "... presents one of the unsolved mysteries of epidemiology ..." and Pollitzer[8] notes that "... this has been the subject of much debate and few conclusions." Others[5] attribute this freedom from plague to the

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displacement of the black rat in European homes by the less-dangerous plague host, the Norway rat.

Epidemic plague reappeared in the last half of the 19th century, apparently originating in Southwestern China, reaching Hong Kong in 1894, and becoming the Third (Modern) Pandemic.[8] From Hong Kong, it spread by cargo vessels to all inhabited continents, newly affecting the Americas. India experienced especially severe epidemics over a 30-year period, resulting in more than 20 million cases and 10 million deaths. In 1894, in Hong Kong, Alexandre Yersin first isolated the causative organism.[9] Studies in India demonstrated that rats were natural reservoirs of infection and also proved the role of fleas in the transmission of the plague bacillus.[5] To combat epidemics of bubonic plague, antisera received extensive study, and various types of vaccines, live attenuated and inactivated whole cell, were made and tested.[10] During World War II, clandestine efforts were made by several combatant nations to weaponize plague, and crude experimental devices were used against some Chinese civilian populations.[1] Curative antibiotic therapy was first demonstrated in the late 1940s and early 1950s using streptomycin, chloramphenicol, and tetracycline.[11,12]

Advances in community sanitation and hygiene have done much to limit contacts of humans with rodents and their fleas; although plague is a widespread zoonosis, disease in humans has in the past 50 years occurred mostly as sporadic cases and clusters, punctuated by occasional limited outbreaks. A notable exception is the prolonged epidemic plague activity that occurred in war-disrupted Vietnam during 1962–1975,[13–16] and a brief resurgence of urban and rural disease in Madagascar in the 1990s.[17]

3. Methodology

3.1. Sources of Morbidity and Mortality Data

Until 2005, International agreements required immediate notification to the World Health Organization (WHO) of the first case of plague in any area previously free of the disease, as well as regular reporting of all confirmed cases. With the adoption and implementation of the updated International Health Regulations (2005), plague is no longer required to be reported by participating states and the WHO will no longer be publishing plague surveillance data.[18] Under the new regulations, however, if plague is consider to "constitute a public health emergency of international concern", it should be reported to the WHO. In addition to the international regulations, individual countries have various regulations governing reporting in their jurisdictions. Global and regional plague surveillance information was published in the WHO *Weekly Epidemiological Record* and in the *Weekly Epidemiological Report* of the Pan American Health Organization up until 2005. For the United States, the Centers for Disease Control and Prevention (CDC) continues to publish annual plague surveillance information in the *Morbidity and Mortality Weekly Report*. Previous WHO-published worldwide figures are thought to be approximately correct, although significant lapses occur; e.g., plague deaths are not routinely reported by some countries, and information on contained outbreaks is sometimes withheld. There is a tendency for underreporting at the onset of an epidemic and overreporting during its course. Sporadic cases are easily missed, especially those that are treated with antibiotics in early illness and fail to develop a typical clinical picture.

Variations in reporting criteria also contribute to unreliable statistics. In the United States, only laboratory-confirmed cases are reported, whereas in some countries, once there is reason to believe an outbreak is in progress, suspect cases are reported with or without laboratory evidence of infection. Further, the vast majority of cases of human plague occur in rural areas of the developing world where surveillance is poor and access to reliable laboratory testing is limited. Information on reported epidemics of plague in India in 1994 was confused by the use of various and nonspecific case definitions and unreliable laboratory confirmation of cases. Consequently, there was overreporting of cases and conflicting statistics, and important epidemiological and etiologic features of this episode are unclear.[19]

3.2. Surveys

Routine microbiological methods are available for the isolation of *Y. pestis* and for serodiagnosis in humans, rodents, and carnivores. Environmental surveys are based on evaluating ecosystems of involved rodent populations, collecting and identifying rodent and rodent flea species and determining *Y. pestis* infection in them, and collecting and testing of carnivore serum for evidence of recent infection.[20–23] These surveys may be helpful in assessing the health risk, in prompting control and prevention measures (e.g., alerting the public, taking measures to control rodent fleas and rodents, and restricting public access to infected areas), and in evaluating the impact of such interventions.

4. Biological Characteristics of the Organism

4.1. Etiologic Agent, *Yersinia pestis*

Yersinia pestis is a gram-negative, nonmotile, nonsporulating, coccobacillus in the family Enterobacteriaceae.

Molecular genetic studies show that it evolved directly from *Yersinia pseudotuberculosis* less than 20,000 years ago.[24,25] It is microaerophilic, oxidase- and urease-negative, and biochemically unreactive. *Y. pestis* is nonfastidious and grows slowly but well on simple bacteriologic media.[26] It grows best at 28°C and a pH of 7.4. When incubated on agar plates, it produces pinpoint colonies at 24 h and 1–2 mm diameter colonies at 48 h. Colonies are gray-white with irregular surfaces that have a hammered metal appearance when viewed under magnification. In broth, the organism grows in clumps that cling to the sides of the culture tube and the broth itself remains clear. *Y. pestis* readily infects laboratory rodents, which are used in specialized laboratories for virulence testing and for isolating the organism from contaminated materials.

4.2. Virulence Factors

Virulence factors are important in the organism's ability to replicate in fleas and mammals.[26,27] Most genes encoding these virulence factors reside on three plasmids, *Pst* (9.5 kb), *Lcr* (~70 kb), and *pFra* (~110 kb), but some are associated with chromosomes. The *Pst* plasmid encodes a plasminogen activator (Pla) and a bacteriocin (pesticin). The temperature-dependent plasminogen activator, a single-bacterial surface protease, is important for survival of *Y. pestis* in fleas. This protease facilitates the formation of a bolus of blood and aggregated bacteria in the flea midgut, which blocks the flea's proventriculus and leads to regurgitation of infective material when the "blocked" flea attempts to feed.

At least six determinants of *Y. pestis* virulence in guinea pigs and in mice have been identified, and it is thought that most of these are important in human infection. Fraction 1 antigen (F1) is contained in the diffusible envelope of the organism and is associated with protection against neutrophilic phagocytosis. Expression of this antigen is temperature dependent; maximum F1 production occurs between 32 and 37°C and does not occur below 28°C. Because the ambient temperature of fleas is typically less than 28°C, *Y. pestis* in the flea gut lacks F1, and organisms inoculated into mammalian hosts by fleas are initially vulnerable to neutrophilic phagocytosis. Those that survive develop in vivo resistance to neutrophilic phagocytosis.[26,28]

Yops, V, and W virulence proteins are known as low-calcium response proteins and are thought to inhibit phagocytosis and to mediate the inflammatory and cell-mediated immune response to infection.[26,27] Pesticin (Pst) and plasminogen activator protease (Psa) are associated with the production of a fibrinolytic factor and a coagulase. The interplay between Pst and chromosomal iron regulatory genes influences hemin storage and iron transport. Strains that are able to utilize exogenous hemin bind congo red in culture and produce pigment. Murine toxin (Ymt) is specific and highly lethal to mice and less so to rats, but it is not known to be toxic in humans. In the flea midgut, Ymt (recently recognized to be phospholipidase D) plays a critical role in protecting *Y. pestis* from a cytotoxic digestion product of blood plasma. The emergence of Ymt did, by adapting the bacillus to fleas, radically separate the natural history of the plague bacillus from that of the enteropathogenic *Yersinia* spp.[28]

In routine culture of *Y. pestis*, some colonies may be found that lack certain virulence factors. Pigment-negative colonies are the most common findings.[29] Laboratory procedures for isolating *Y. pestis* by inoculating laboratory animals may select against isolates that lack certain virulence factors. Testing for these factors should be done on fresh isolates, because laboratory manipulation, particularly prolonged incubation at 37°C, may result in loss of virulence.

4.3. Geographic Biotypes

Biotypes of *Y. pestis* based on nitrate reduction and ability to ferment glycerol were described by Devignat.[30] These biotypes are not related to virulence, but were thought to be of epidemiological interest as markers of evolving lineages. The Orientalis biotype spread from East Asia to many areas of the globe in the Third Pandemic, including the Western Hemisphere. It is characterized by its ability to reduce nitrate and an inability to ferment glycerol. Variety Mediaevalis, found only around the Caspian Sea, does not reduce nitrate but ferments glycerol. Variety Antigua, present in Southeastern Russia, Central Asia, and parts of Africa, both reduces nitrate and ferments glycerol. Although ribotyping studies of various plague strains generally support the biotyping distinctions,[31] results of recent molecular genetic studies contradict neat historical compartmentalizations.[32,33]

4.4. Flea Vectors

Fleas are the only known arthropod vectors of plague. They typically leave a dead host as its body temperature drops and seek a warm-blooded replacement, enhancing spread of infection. Many flea species are strongly host specific; others, such as *Xenopsylla cheopis* (the oriental rat flea), which is the principal vector of urban epidemic plague, are promiscuous in their host selection. Other important vectors of plague to humans are *X. astia, X. braziliensis, X. vexabilis, Nosopsyllus fasciatus*, and in the United States, the wild rodent flea, *Oropsylla montana*.[8,20]

When *X. cheopis* ingests blood containing *Y. pestis*, bacilli multiply and form an alimentary canal blockage at the level of the proventriculus. The observation that epidemics of bubonic plague waned when ambient temperatures exceeded 28°C led to studies demonstrating that *X. cheopis* enzymatically lyse their intestinal blockage at temperatures above 28°C. Cleared fleas could feed without regurgitation and were no longer efficient vectors.[34] Newer studies using both *X. cheopis* and *O. Montana* fleas have demonstrated, however, that the fleas can transmit *Y. pestis* during the early phase of infection without an intestinal blockage and thus challenge the need for intestinal blockage for effective transmission and spreading of plague.[35,36] The vector competency of fleas is evaluated by comparing them to the prototypic, highly efficient vector, *X. cheopis*.[21,37]

4.5. Rodent Reservoirs

Of the more than 200 species of rodents found to be naturally infected by *Y. pestis*, many are unimportant in maintaining zoonotic cycles of infection and only a few pose significant risks to humans. *Rattus rattus*, the domestic black rat (house rat, roof rat), is the most dangerous. It has a cosmopolitan distribution, lives in close association with humans (nesting in attics, wall spaces, etc.), is a vigorous breeder, is a host favorable to *X. cheopis*, and typically develops fatal septicemic plague. In epizootics, rats serve as efficient amplifying hosts of *Y. pestis*. "Rat-fall" refers to the observable die-off of affected rat populations and is a forewarning of epidemic plague. However, rat populations that have experienced extended periods of plague activity may become relatively resistant to infection, and rat-falls may cease even though transmission of *Y. pestis* continues. *R. norvegicus* (Norway rat, sewer rat) is an aggressive territorial competitor of the black rat. It is a burrowing rodent that lives peridomestically and frequents cellars, drains, crawl spaces, and foundation areas of buildings. In rural areas, the Norway rat may be an efficient vectorial link between the wild rodents and the more domestic black rat. When wild rodent plague is contiguous with rat and oriental rat flea populations, the potential for epidemic plague is great.[8]

Important noncommensal sources of plague throughout the world include marmots, gerbils, and ground squirrels in Northern Asia; gerbils and ground squirrels (susliks, sisels) in Central Asia and the Near East; gerbils and bandicoot rats in India; the Polynesian rat (*Rattus exulans*) and shrews in Vietnam; *R. exulans* in Indonesia; gerbils, grass rats, and some species of multimammate mice in Africa; ground squirrels (*Spermophilus* spp.), prairie dogs (*Cynomys* spp.), and chipmunks (*Tamias* spp.) in the United States; and guinea pigs (*Cavia* spp), rice rats (*Oryzomys* spp.), and cotton rats (*Sigmodon* spp.) in South America.[8,20,38]

5. Epidemiology

5.1. Occurrence of Human Plague

During 1989–2003, a total of 38,359 human cases of plague was reported to the WHO from 25 countries (mean of 2,557 cases per year) (Table 1).[39] The reported case fatality ratio was 7%. More than 80% (31,273) of the total number of cases were reported from African countries, about 14% (5,449) from Asia, and the rest from the Americas (1,637). Countries reporting the highest number of cases were Tanzania, Zaire, and Madagascar in the African region; Vietnam and Myanmar in Asia; and Peru in the Americas.

Table 1 Reported Cases of Plague in Humans by Country, 1989–2003

Continent	Country	No. of cases	No. of deaths
Africa	Algeria	11	1
	Botswana	173	12
	Kenya	44	8
	Madagascar	13,148	1132
	Malawi	907	16
	Mozambique	2387	28
	Namibia	2012	80
	Tanzania	5249	335
	Uganda	821	130
	Zaire	4935	616
	Zambia	1169	29
	Zimbabwe	417	34
	Total	31,273	2421
America	Bolivia	37	6
	Brazil	105	0
	Ecuador	14	14
	Peru	1380	60
	United States	101	10
	Total	1637	90
Asia	China	519	52
	India	892	58
	Indonesia	6	0
	Kazakhstan	24	7
	Lao People's Democratic Republic	10	0
	Mongolia	115	36
	Myanmar	761	6
	Vietnam	3122	175
	Total	5449	334
World total		38,359	2845

Recent outbreak activity has been reported from Democratic Republic of Congo, India, Algeria, Zaire, Madagascar, Mozambique, Zambia, Malawi, Indonesia, and Educador. India, in 1994, reported a total of 876 bubonic and pneumonic plague cases with 54 deaths, but the number of confirmed cases was much less.[19,40]

In the United States, 129 confirmed cases of plague in humans were reported during 1989–2006 (average 7 cases per year). Of these cases, 78% occurred in the Southwestern states of New Mexico, Arizona, and Colorado; 9% were reported from California; nine other western states reported small numbers of cases, and one case was imported (CDC, unpublished data). Most likely modes of transmission were determined for 71 of these cases and included flea bite ($n = 27$; 38%) and direct contact with infected animals ($n = 44$; 62%). Of the 44 persons thought to be infected by direct contact with animals, just under half [17] reported exposure to an infected domestic cat. The overall case-fatality ratio was 10%. In the United States, most cases of plague occur in the summer months when rodent and rodent flea activities are greatest. The majority of cases, especially those in the southwestern states, are acquired in the environs of the patient's residential property [41,42]; less often, plague in the United States is acquired by persons working or recreating in natural areas remote from their places of residence.

5.2. Contagiousness

Uncomplicated bubonic plague is not contagious and patients do not place their family, other social contacts, or attendants at risk. Household members, however, may be at risk of exposure to the same zoonotic source as the index case. Most plague exposures occur in rural foci, where cases are more likely to be sporadic than epidemic. When a bubonic or septicemic plague patient develops secondary plague pneumonia, person-to-person transmission becomes possible but rarely occurs; no person-to-person plague cases have occurred in the United States since 1924, even though 67 pneumonic plague cases have been recorded since then, including six with primary pneumonic plague acquired from domestic pets (five cats, one dog) with respiratory plague (CDC, unpublished data). The presence of *Y. pestis* in the throats of presumably healthy persons has been described,[43] but there are no data to suggest that such "carriers" play any role in the transmission of the infection. Any draining buboes or other cutaneous lesions of plague patients should be considered infectious, but experience indicates that risk of infection by direct contact with exudates is minimal.

The plague bacillus does not survive well in the environment. Temperatures above 40°C and desiccation are rapidly lethal to the organism, and airborne organisms have a short-survival period. Pneumonic plague is transmitted by respiratory droplet, and *Y. pestis* does not become truly aerosolized in an infectious state. Respiratory droplet spread occurs only when there is close, face-to-face contact (<2 m).

5.3. Geographic Distribution

Countries reporting human plague are noted in Table 1.[39] The probable distribution of active enzootic plague foci throughout the world is shown in Figure 1.

5.4. Environmental Factors

Ambient temperature and humidity regulate rodent host populations as well as the survival and continued infectiousness of their fleas. Bubonic plague epidemics have occurred most notably in temperature ranges of 10–26°C associated with a high-relative humidity.[8,34,38] These conditions are most favorable for plague epizootics in rats. On the other hand, enzootic wild rodent plague foci are most often associated with semi-arid regions.[20,21,38] In tropical environments, outbreaks tend to occur at the end of the dry season, when the temperature falls and humidity rises. In India the rainy season differs between the northern and the southcentral plague foci, so that no generalization as to time of year for greatest plague activity can be given for the country as a whole.[8] In Vietnam, human plague incidence was found to be sensitive to slight variations in temperature, relative humidity, and vapor pressure.[34,37,44]

Pneumonic plague epidemics have occurred in winter in circumstances of high-relative humidity and overcrowding; this was most dramatically demonstrated in Manchuria during two major epidemics in the early 20th century, when tens of thousands of persons died in epidemics of pneumonic plague.[6] The California urban plague outbreaks in 1919 and 1924, and the recent descriptions of pneumonic plague in highlands of Madagascar, are examples of limited transmission of pneumonic plague in moderate climates.[17,45]

The principal environmental determinants of risk to humans of rat-borne plague are the underlying sanitation and the conditions of homes and places of work that support rat populations with food and harborage. Populations of wild burrowing rodents fluctuate with the availability of edible vegetation.

5.5. Demographic Factors

Demographic differences in rates of disease can be explained by exposures associated with place of residence, cultural and socioeconomic factors, occupational and recreational activities, and other behavioral factors.[46] Poverty,

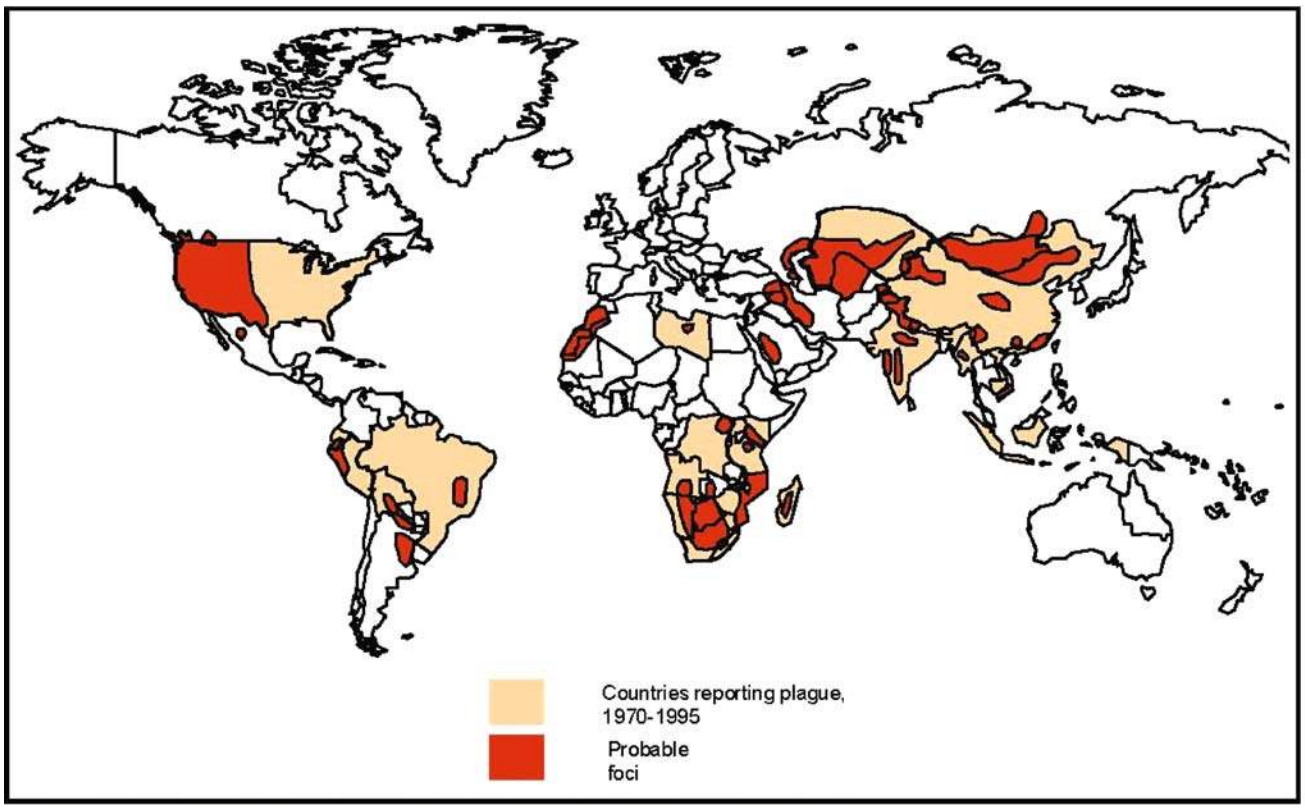


Figure 1. Distribution of natural foci of plague. Compiled from WHO, CDC, and other sources.

crowding, and poor sanitation are risk factors for rat-associated plague.

5.6. Occupation and Recreation

Certain occupational and recreational activities are associated with an increased risk of exposure to infected fleas. Persons who work or recreate in areas where there are large populations of burrowing rodents such as prairie dogs and ground squirrels, including hunters, trappers, naturalists, pet owners, and campers, may be at an elevated risk of infection.[47,48] This is important in explaining the relatively large numbers of cases that occur among Native Americans in the southwestern United States.[48,49]

In Mongolia, and in some high-elevation areas of Central Asia, marmot hunting and skinning is an important risk factor for plague.

5.7. Occurrence in Different Settings

When family or household clusters of bubonic cases occur, the dates of disease onset usually occur within days of each other. Where multiple cases have occurred within a larger community from a single-epizootic source, dates of onset are usually separated by weeks and involve multiple and geographically separated households. Pneumonic plague patients pose a threat to immediate contacts including family members, friends, and medical personnel. Travel during the incubation period of plague, including long-distance air travel, has been described in the United States as peripatetic plague[50,51] and occurred most recently in 2002, when two persons became ill while traveling in New York City.[52] Molecular subtyping showed that the patient isolates were indistinguishable from rodent isolates obtained near their home in New Mexico. Some traveling patients developed secondary pneumonic plague and were contagious for variable periods before being isolated and treated. Fortunately, the risk of pneumonic transmission is high only when there is a productive cough and close face-to-face exposure.[17,53,54] Disruptions of war have often been associated with plague epidemics. Invading or retreating troops have introduced the disease by transporting infected rodent hosts or their fleas in shipments of grain, other food stuffs, and material.[8,38]

Movement and crowding of refugees provide the opportunity for pre-existing plague to become epidemic.[55]

5.8. Socioeconomic Factors

In rat-borne disease, persons with low incomes are generally the most affected. Factors include poor-quality housing that allows ready access to and harborage for rats; housing located near docks, warehouses, or granaries; crowding; and poor environmental hygiene and sanitation. In the United States, cases arise primarily from wild rodent epizootic sources and all socioeconomic groups are affected. The affluent build expensive homes in rural areas with the intention of retaining as much of the natural habitat as possible, sometimes in the midst of an enzootic plague focus. Decorative rock walls form ideal "rocky outcroppings," the principal habitat of the rock squirrel (*Spermophilus variegatus*), an important plague host in the Rocky Mountain region. On the other hand, some persons who live in close proximity to enzootic plague foci encourage rodent encroachment (especially by wood, or pack, rats) by providing peridomestic food and harborage in the form of garbage, junk piles, old cars, tires, and wood piles.[47] Prairie dogs may invade overgrazed pastures surrounding or adjacent to human dwellings, which has been especially problematic on Native American lands in the Southwestern United States and increasingly at the interface of the plains and Rocky Mountain foothills in Colorado.

6. Mechanisms and Routes of Transmission

The sources of *Y. pestis* infection of humans are bites by infected fleas; direct contact with the blood or tissues of infected animals captured, hunted, or found dead; persons or pets with plague pneumonia; ingestion of raw or undercooked meat from an infected animal; and rarely the mishandling of plague cultures in the laboratory. Infection can take place through the skin, the respiratory tract, the conjunctiva, the oropharyngeal mucosa, and the digestive tract. Infection by direct conjunctival or mucous membrane exposure is of concern to persons caring for patients with plague pneumonia, laboratorians, and persons who dissect or butcher infected animals.

Entry of the plague bacillus through the respiratory tract may result in two clinical patterns, depending on the size of aerosolized particles introduced, either pharyngeal or pneumonic plague. Experiments in guinea pigs showed that bacilli contained in large-particle sprays were most likely to lodge in the oropharynx resulting in pharyngeal plague and secondary septicemic plague, while smaller particles initiated bronchopneumonia; in experiments with larger animals such as monkeys, inhalation either of single-organism clouds or infectious particles 12 μm in diameter produced lobar pneumonia.[56] Transmission of pneumonic plague among humans is thought to occur through respiratory droplets as transmission by droplet nuclei has not been demonstrated.[53,54]

Domestic cats develop bubonic plague with submandibular and/or cervical lymphadenopathy and associated ulceration, and abscesses, or they can develop pharyngeal or pneumonic plague as a result of ingesting infected rodents.[57,58] Humans can acquire various forms of plague from cats by inhaling infective respiratory particles, by skin or mucous membrane contact with infectious exudates or secretions, or by being bitten or scratched. Ingestion of undercooked infected wild rodents (especially marmots and prairie dogs) and of infected camel and goat meat has resulted both in sporadic cases and limited outbreaks of human plague, which manifest as plague pharyngitis, septicemia, and meningitis.[59]

7. Pathogenesis and Immunity

7.1. Pathogenesis

Following the bite of an infected flea or other cutaneous introduction, local *Y. pestis* proliferation ensues but is often not apparent; a small papule or vesicle containing a clear or hemorrhagic fluid (a phlyctenule) sometimes occurs at the site of inoculation and rarely a crater-like ulcer mimicking that of tularemia develops at the site.

From the site of inoculation, organisms travel by way of the lymphatics to regional nodes. The disease may be contained at this level as in simple bubonic plague, or localized infection may be followed by bloodstream invasion with intermittent bacteremia or septicemia. Septicemic plague may occasionally develop with no apparent local lymphatic involvement (primary septicemic plague). Some of these patients are thought to have occult involvement of intra- or retro-peritoneal nodes. Bloodstream dissemination may lead to secondary plague pneumonia, plague meningitis, multiple lymphadenopathy, and involvement of other organs, such as the liver, spleen, and rarely the eye.

The principal direct pathogenic effect of invasion is acute local inflammation and hemorrhagic necrosis of affected tissue. This will trigger the release of cytokines and other inflammatory mediators that in turn can activate the complement cascade. Systemically, the patient responds with fever and endotoxemia. A septic shock syndrome can

develop leading to refractory shock, organ failure, acute respiratory distress syndrome, and disseminated hemorrhagic manifestations, including petechiae, ecchymoses, and frank bleeding.[60]

7.2. Incubation Period

The incubation period of bubonic plague is generally 2–6 days,[8] and is typically 3–5 days for pneumonic plague, but shorter and longer intervals have been reported.[13,14,61]

7.3. Immunity

Susceptibility of rodents to *Y. pestis* varies from one species to another. Species that are moderately resistant and have a low mortality are important in maintaining plague in a focus and are termed *enzootic* or *maintenance hosts*. They usually coexist with susceptible rodent species that experience a high mortality from infection (epizootic, amplifying hosts). In the United States, inherent species susceptibilities range from highly resistant (kangaroo rats) to highly susceptible (prairie dogs).[22] Studies of the natural resistance in rats from a plague-free area showed that some survivors of a small challenge inoculum that developed antibody responses survived a rechallenge with a similar or larger inoculum, while most of those that did not develop antibodies died.[62]

Most humans develop antibody responses to infection with *Y. pestis*.[63] In a recent CDC review (unpublished) of 89 confirmed plague cases in the United States, only 4 (4.5%) cases did not develop antibodies to F1 of *Y. pestis*. Asymptomatic infection in humans appears to be rare, but some evidence for this was found in Madagascar by detecting plague antibodies in persons from whom no history of illness could be elicited.[64] On the other hand, serosurveys of 716 community and household contacts of cases living in plague-enzootic areas of the United States and 167 residents of two villages in Indonesia during a rat-borne plague epidemic there revealed only four seropositive samples. One, with a titer of 1:128, was a missed bubonic plague case who had onset of illness 3 weeks prior to the survey; one was a Vietnam veteran with a titer of 1:32 who had been previously immunized; two were residents of an Indonesian village with titers of 1:16 and 1:32, respectively, and who had no history of recent illness or of vaccination.[65] This suggests that subclinical infection was rare to nonexistent in those cases.

Persons previously immunized with formalin-inactivated plague vaccine USP may develop titers against *Y. pestis* that persist for decades. Prior plague vaccination may confound results of serological testing if a history of vaccination is not considered.

The degree of protection provided by plague antibodies in humans is not fully known. Experience in Vietnam suggests that natural infection may not produce complete immunity.[64] And, although the inactivated plague vaccine available in the United States in the past was shown to be an efficient immunogen, its protective efficacy was never tested in controlled clinical trials. Comparisons of immunologic responses to vaccination by humans and animals and field experiences with vaccinated military personnel provide indirect evidence that the inactivated vaccine was protective against bubonic plague in humans.[66] Recently developed recombinant F1 and V antigen vaccines currently undergoing clinical trials are reported to be protective in mice and highly immunogenic in humans.[67]

8. Patterns of Host Response

The clinical response of humans to *Y. pestis* infection is diverse, ranging from mild, ambulatory bubonic plague to rapidly progressing septicemia with bleeding, shock, multiple organ failure, and death.[68–72]

8.1. Clinical Features

The clinical forms of plague that arise from primary exposure include bubonic, septicemic, pneumonic, pharyngeal, and possibly gastrointestinal plague; secondary manifestations that result from lymphatic or hematogeneous spread to other organ systems include multiple lymphadenitis (sometimes involving intra-abdominal and thoracic lymph nodes), plague pneumonitis, meningitis, abscess formation in the liver or spleen, and endophthalmitis.[16,68,72]

Two to six days after exposure, bubonic plague patients typically experience sudden onset of illness characterized by severe malaise, headache, shaking chills, fever greater than 38°C, and pain in the area of affected regional lymph nodes, which may not be palpably enlarged at this stage. Progression of symptoms is usually rapid with the affected nodes (buboes) often becoming excruciatingly tender and painful as they enlarge. Definition of small to moderately enlarged buboes may be obscured by extensive perinodal edema, erythema, and induration. During the course of the first 3–5 days of illness, the patient may, if not treated, develop gram-negative sepsis, endotoxin-induced systemic inflammatory response syndrome, consumptive coagulopathy, subcutaneous and widely disseminated hemorrhages, septic shock, multiple organ failure, adult respiratory distress syndrome (ARDS), mental confusion, and death.[68–72] Buboes may or may not suppurate.

In primary pneumonic plague, usually within 3–5 days after exposure, the patient experiences a chill followed by fever, splinting of the chest, and cough with production of a thin, serosanguinous sputum. Segmental pulmonary consolidation may occur, manifested by dullness to percussion and decreased breath sounds. A more diffuse interstitial pneumonitis may develop and progress rapidly. Not infrequently, auscultatory findings are surprisingly minimal compared to what is seen by roentgenography. Lobar pneumonia, abscess formation, and cavitation are unusual complications of pulmonary infection. If efficacious antibiotics and aggressive supportive care is not provided within 18 h or so of onset, primary pneumonic plague almost always follows a fulminating course and death within 2–3 days.

Pulmonary infection is a serious public health concern, since respiratory plague is transmissible from person to person. Secondary plague pneumonia occurs more frequently than primary plague pneumonia and is thought overall to be less contagious than the primary form. At time of contagiousness, the secondary pneumonia patient usually has been severely ill for 2 or more days with sepsis, is less capable of mustering a vigorous cough, and most often produces sputum that tends to be thick, mucopurulent, and tenacious. In comparison, the primary pneumonic plague patient is more likely to produce a thin, watery, dispersable serosanguinous or bloody sputum early in the disease and to cough with more vigor.

8.2. Differential Diagnosis

When bubonic plague occurs in epidemic form, it is readily diagnosed. Conversely, the diagnosis of an isolated case is often missed, particularly in the early stage of illness. Plague should be considered in any patient presenting with acute onset of fever, lymphadenitis, and a history of residence or travel in a known plague area. Other causes of lymphadenopathy, however, must be investigated: gram-positive bacteria are probably the most common cause, and a Gram's stain of a bubo aspirate should quickly differentiate between gram-positive cocci and gram-negative bacilli. The list of differential causes of lymphadenopathy is long, and plague cases have been misdiagnosed as cat scratch fever, lymphoma, appendicitis, and incarcerated inguinal hernia, to name a few. Bubonic plague and tularemia may be confused in areas where both diseases occur.[73] A skin lesion at the site of inoculation may be seen in both diseases. From a clinical standpoint this may not be a critical distinction, since both respond to the same antibiotic therapy; however, for public health and epidemiological reasons, a missed case of plague is potentially serious. The marked tenderness and pain of affected lymph nodes of bubonic plague patients present a sharp contrast to the relatively milder discomfort that occurs with most other infectious causes of lymphadenitis.

Pneumonic plague may be confused with severe acute respiratory illness, including ARDS, from other causes. In the United States, pneumonic plague has been confused with hantavirus pulmonary syndrome and vice-versa. A history of recent exposure to a person with severe fatal pneumonia, or to a pet cat with an acute febrile illness, cervical or submandibular lymphadenopathy or abscess, or bloody respiratory discharge[59,74,75] should prompt the clinician to consider plague. It is critically important to obtain a careful and thorough history of animal exposures from every suspect plague patient.

When plague is considered, an examination of bubo aspirate or sputum by Gram's stain and by a polychrome stain should guide the clinician in choosing an appropriate antibiotic. When plague is suspected and other causes of illness cannot be implicated, plague therapy should be started pending the outcome of definitive testing. In patients with suspected bubonic plague who do not yet have a microbiologic diagnosis, it may be prudent to initially include therapy for gram-positive causes of lymphadenopathy; gram-positive bacteria grow rapidly on culture so that a microbiological diagnosis can usually be made within 12–24 h.

8.3. Laboratory Diagnosis

8.3.1. Clinical Specimen Collection and Staining. When plague is suspected, laboratory diagnostic specimens should be collected immediately, chest radiographs obtained, and the patient begun on appropriate antibiotic therapy pending microbiological confirmation. Specimens routinely collected include blood on all patients, lymph node aspirates from those with suspected buboes, sputum samples or tracheal washes from those with suspected respiratory plague, and cerebrospinal fluid from patients with suspected meningitis.

Blood is aseptically collected for culture, for preparation of blood smears on glass slides, and for leucocyte counts. For bubo aspiration, a small, sterile syringe containing a few milliliters of sterile saline is inserted into the central part of the suspect lymph node and an attempt is made to aspirate fluid. If this is not successful, the saline is injected and aspirated. A portion of the aspirate is used to make films on two or more glass slides for routine staining, and at least two slides for direct fluorescent antibody (FA) testing. Slides for staining are prepared by placing drops of the aspirate on glass slides, which are then air-dried and lightly heat-fixed. For routine staining, one slide should be stained by Giemsa or Wayson's method and one by a Gram's stain. To identify *Y. pestis*, specimens of blood, sputum, tracheal washes,

and cerebrospinal fluid should be similarly stained. Under light microscopy, *Y. pestis* is a plump gram-negative bacillus that, with polychrome stains, typically shows a bipolar, closed safety-pin configuration. Fluorescence-stained organisms are identified using darkfield microscopy.[76]

8.3.2. Cultural Isolation of *Y. pestis*. Cultural isolation of *Y. pestis* is important for early confirmation of infection. If the patient's condition allows, at least three blood cultures should be taken over a 45-min period immediately after the diagnosis of plague is suspected and before antibiotic treatment is begun. Blood and other materials, such as bubo aspirates and sputum, should be inoculated onto media such as sheep blood agar, brain–heart infusion (BHI) broth, or MacConkey or deoxycholate agar; when immediate culture is not possible, specimens may be placed in Cary–Blair transport medium. Confirmation of isolates as *Y. pestis* is made by specific bacteriophage lysis.[76]

8.3.3. Serological Diagnostic Methods. The standard serodiagnostic method is the passive hemagglutination (PHA) test utilizing *Y. pestis* F1 antigen. The specificity of a positive antibody–antigen reaction should be validated by hemagglutination inhibition (HI) testing. Acute and convalescent serum specimens should be taken. Serum samples taken 3–4 weeks after onset of symptoms will confirm more than 95% of cases. PHA antibodies tend to remain at high titer ($\geq$64) for months and may persist at low titer for years or for life. An enzyme-linked immunosorbent assay to detect the F1 antigen in serum or blood clot is quite sensitive; however, this procedure does not recognize all blood-culture-positive patients.[77] A complement-fixation test with F1 antigen is comparatively insensitive, especially in early infection. Latex particles coated with F1 antigen have been used successfully in direct and passive agglutination tests, although problems in sensitivity and specificity have been reported.[76]

8.3.4. Rapid Detection Tests. Antigen capture ELISA procedures, polymerase chain reaction assays, and handheld immunochromatographic detection tests for rapid early detection of the F1 antigen have been developed and are being evaluated. The handheld devices permit diagnostic testing of clinical materials at the bedside, even under primitive conditions.[78]

8.3.5. Reference Diagnostic Testing. Whenever the diagnosis of plague is considered in the United States, materials should be sent to clinical laboratories experienced in testing for plague. In addition, the material should be sent to the State Health Department and the CDC, Fort Collins, Colorado 80521, for microbiological confirmation. The Local and State Health Department should be contacted by telephone for reporting of a suspected case, for assistance in diagnostic testing (including the confirmation of an isolate as *Y. pestis*), and for other consultations. The CDC can also be contacted for additional information at 970-221-6400.

8.3.6. Diagnostic Categories. Based on clinical and laboratory findings, cases of plague can be categorized as suspect, presumptive, or confirmed. A *suspect case* is one in which the patient has disease manifestations suggestive of plague as well as consistent epidemiological features. A *presumptive case* is one in which the patient has compatible clinical manifestations and supportive laboratory findings, including gram-negative, bipolar bacilli seen in stained smears of a bubo, blood or sputum; a single seropositive test result for *Y. pestis* infection; PCR detection of *Y. pestis* in bubo, aspirate, or sputum; or F1 antigen detection in bubo, aspirate, blood, or sputum. A *confirmed case* is one in which there is a fourfold or greater change in *Y. pestis* antibody titer between acute- and convalescent-phase serum samples, or in which a bacterial culture of suspected *Y. pestis* is lysed by the uniquely specific bacteriophage used for this purpose. In endemic areas when no other confirmatory test can be performed then a case having a positive rapid diagnostic test using immunochromatography to detect F1 antigen is also considered a confirmed case.[79]

9. Control and Prevention

9.1. General Concepts

The major objective of control is reduction of bites by infective fleas. Education of the general public and health professionals in areas where plague occurs is the first line of defense in plague prevention. Risk factors in those areas must be publicized, especially at the outset of known plague seasons. Physicians, veterinarians, and the public must be alerted to the possibility of plague and know its signs and symptoms. Persons in endemic areas need to know the potential danger from rodent die-offs and should report changes in rodent activity to public authorities. They should also be educated on the need to control fleas on pet dogs and cats; to properly store grains and other foodstuffs that may attract rodents to home, work, or play areas; to rodent-proof homes; and to take appropriate action when there is severe illness, death, or disappearance of a pet cat. Hikers, campers, and others who take part in outdoor recreational activities in areas where plague occurs should (1) avoid handling of sick or dead animals, (2) avoid rodent nests and burrows, (3) use insect repellents containing *N*,*N*-diethyl-m-toluamide (DEET) on skin and repellents or appropriate insecticidal sprays on clothing, and (4) treat accompanying pets with insecticides. Hunters should use gloves when handling dead animals.

During epidemics of bubonic plague, flea control must precede or effectively coincide with rodenticiding. Death of rodent hosts without prior flea control will deprive fleas of their normal hosts, forcing them to seek alternate sources of blood meals, including humans, and greatly increasing the risk of human exposure. Appropriate insecticides should be applied to rodent burrows, nests, rat runs, and other sites where rodents are active. This should be done under the supervision of persons qualified in pest control. Tests should be set up to determine whether the target fleas are susceptible to the insecticides considered for use and to evaluate the effectiveness of flea control efforts.[20,21] After reduction of the flea population, trapping or poisoning of rodents may be appropriate in certain situations. Killing of commensal rodents will provide no benefit unless rodent-proofing of houses and elimination of food and harborage are also undertaken. Rodents will quickly move into the vacated spaces and may aggravate an epidemic situation, since in-migrating rodents are apt to be young and more susceptible to *Y. pestis* than the removed population. During rat-borne epidemics, bubonic plague patients, their clothing, and other effects may still be carrying infected fleas, and decontamination measures may be needed.

Plague is potentially a quarantinable disease covered under international regulations.[80] Following reports of outbreaks of plague in India in 1994, international and national health authorities became involved for the first time in many decades in reviewing, interpreting, and implementing regulations concerning the threat of international spread of plague.[39,81,82] Travel and trade with India was severely disrupted, and the outbreaks were estimated to have cost India more than a billion dollars lost in tourism, commerce, and investment values, disruption of services, and prevention and control efforts. This experience will hopefully lead to improved understanding by public health authorities of the true risks for international spread of plague and the use of more appropriate control measures in future similar occurrences.

Pneumonic plague patients may incubate very large numbers of organisms, but appropriate therapy almost always results in a marked decrease in contagiousness in a matter of hours.

9.2. Antibiotic Treatment

Early treatment of cases with effective antibiotics is important in preventing complications and death, and a principal measure to prevent respiratory spread of infection. Standard antibiotics used in treating plague are doxycycline, streptomycin, and chloramphenicol.[83,84] Increasing numbers of plague patients in the United States are being treated with gentamicin, and evidence is accumulating that this is a safe and efficacious antibiotic for this disease.[85,86] Ciprofloxacin cures plague in laboratory animal experiments and has been recommended as an alternative for both treatment and prophylaxis when large numbers of persons are involved in a bioterrorism exposure.[1,2] A single isolate of *Y. pestis* from a bubonic plague patient in Madagascar was found to be resistant to multiple antibiotics usually used to treat plague, and this resistance was plasmid mediated.[87]

Patients with pneumonic plague should be considered contagious and placed on respiratory isolation until at least 48 h of effective antibiotic therapy and clinical improvement has taken place.[1]

9.3. Antibiotic Prophylaxis

In circumstances where infection results from rat and flea infestations of houses, drug prophylaxis may be advisable for members of the household and possibly adjacent households, depending on the environmental circumstances. Persons in close contact with infectious pneumonic plague patients during a previous 6-day period should be given postexposure treatment for 7 days. This is both to protect the individual and to prevent further spread. Those who decline drug treatment should be placed under surveillance for 7 days since last exposure. Short-term antibiotic prophylaxis may be recommended in unusual situations for travelers who cannot avoid plague epidemic areas and for health-care workers caring for suspected plague cases in an epidemic situation.

Recommended prophylactic drugs include the tetracyclines for adults and older children and sulfonamides in children younger than 9 years of age; chloramphenicol is also effective.[83]

9.4. Immunization

There are currently no plague vaccines available in the United States. In some countries, living "avirulent" *Y. pestis* organisms are used as immunogens. Studies in the United States have confirmed that such preparations are effective in experimental animals, but none of these is commercially available and their safety and efficacy in humans have not been adequately tested.[10,67,88] Beginning in 1942, a formalin-killed suspension of whole virulent plague bacilli with phenol added as a preservative was used in the United States to vaccinate military personnel and some other persons considered to be at high risk of infection, such as laboratory workers. Different strains of *Y. pestis* were tried, the quantity of organisms per dose was varied, and the dose schedule was altered repeatedly.[88,89] It was not known

which antigens were protective in humans, although recombinant F1 and V antigens were shown to be protective in experimental animals.[66,90,91] The evidence for protection in humans was indirect and weak. In Vietnam during 1961–1971, there were only eight plague cases in vaccinated Americans, while many thousands of cases occurred in the indigenous peoples. US military personnel were undoubtedly exposed to the fleas that transmit plague, since they experienced a relatively high incidence of murine typhus, which is also transmitted by *X. cheopis*.[15,66] Acute and convalescent serum specimens were collected from 59 Americans who had received killed plague vaccine and who experienced clinical and serologically proved murine typhus. F1 antibody was found in the initial serum samples of 39 individuals, and this was attributed to prior vaccination. However, a finding of rising titers or seroconversion in seven patients was interpreted as evidence of subclinical *Y. pestis* infection in persons previously immunized. Experts concluded that the vaccine protected military personnel against bubonic plague during both World War II and the Vietnam conflict.[66] There was no evidence that it protected against pneumonic plague. This vaccine, which had only limited utility, is no longer manufactured in the United States and is not available commercially.

Due to increasing concern that *Y. pestis* could be used as a weapon of terrorism, interest has been renewed in developing a new and improved generation of plague vaccines using molecular methods. The principal approach has been to generate subunit vaccines using a combination of recombinant F1 and V antigens.[67,91,92] These vaccines generate protection by stimulating IgG responses as well as T cell memory. Various recombinant F1 + V combined or fusion subunit vaccines are in advanced stages of development; one formulation has demonstrated both safety and immunogenicity in phases 1 and 2 clinical trials.[92]

9.5. Public Health Management of the Use of Plague as a Weapon of Terrorism

Guidelines on medical and public health management of plague as a biological weapon have been published,[1,2] and various national, state, and local plans are available for responding to bioterrorism and other similar public health emergencies. The critical first step is early detection and recognition that an attack using *Y. pestis* has occurred. Although biosensors are being deployed in special locations and at special events, it is most likely that the first indication of an occult event would be the occurrence of an unusual cluster of cases of severe respiratory illness. Case definitions for illness need to be constructed and disseminated for use by clinicians and epidemiologists for early identification of ill suspects. Since it is expected that the plague organism would be used as an aerosol, heightened surveillance should be instituted for severe respiratory illness, including plague pneumonia and plague pharyngitis. Measures should be taken to isolate and treat all suspected cases at the earliest possible time after onset of illness. Proper infection-control measures would include management of patients under respiratory droplet precautions, with masking of persons who might be at high risk of infective exposures, such as patient care-givers, transporters, and interviewers. The population at risk must be quickly delimited and steps be taken to identify and treat prophylactically during the incubation period those persons who may have had infective exposures at the time of the attack or through direct exposure to infective patients, such as family members and others who have had close and unprotected contact with patients with respiratory symptoms. Treatment should be standardized and taken into account of the possibility of use of organisms with bioengineered antimicrobial resistance. Clear lines of authority and decision-making are critical for a well-organized response, as are advance communications systems and distribution of information necessary to maintain order and effective action.

10. Unresolved Problems

The dynamics of enzootic plague and factors responsible for its introduction, maintenance, spread, or disappearance are not well known. Enzootic foci are widespread and readily available for study, but funding of field research is inconstant. Enzootic plague seems to be dependent on a complex interplay between moderately susceptible and resistant rodents, their fleas, modifications in the landscape, and climate. In the early part of this century, plague was introduced repeatedly into some areas without becoming established in wild rodents; in some areas, enzootic foci have advanced (Western United States) and in other areas they have apparently regressed (India, Indonesia). There are currently no feasible means of eliminating enzootic foci in wild rodent reservoirs.

Despite important advances in understanding molecular factors in the virulence of *Y. pestis*, many questions remain unanswered on pathogenicity of infection in humans. It is not known how plague endotoxin and other virulence factors sometimes cause an irreversible immunologic cascade and death when antibiotic treatment is delayed. Further, clinical trials are needed to examine treatment safety and efficacy of newer antibiotics as compared with streptomycin, tetracycline, and chloramphenicol. The relative rarity of plague in the world today, and the remoteness of the areas where it is

most likely to occur, make it difficult to directly investigate the pathogenesis and treatment response in humans.

Finally, the considerable recent concern over the potential use of *Y. pestis* as a weapon of terror has raised important research questions related to how it might be delivered as a weapon, its public health impact under various circumstances of delivery, the means of early detection and characterization of the agent, and how targeted populations could best be protected, including the use of prophylactic and curative antibiotics and the role of a newer generation of vaccines now under development. The potential for use of an agent resistant to antimicrobials and possibly to vaccines and means to counter this are subjects of study.

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CHAPTER 29

Pneumococcal Infections

Keith P. Klugman and Charles Feldman

1. Introduction

The pneumococcus (*Streptococcus pneumoniae*) is the major cause of bacterial pneumonia and meningitis worldwide. In his classic book entitled *The Biology of the Pneumococcus*, published in 1938, Benjamin White[287] listed 19 different names applied to the pneumococcus between 1897 and 1934, the year the designation *Diplococcus pneumoniae* Weichselbaum was approved in the fourth edition of *Bergey's Manual*.[25] In the eighth edition of *Bergey's Manual*,[47] the microorganism was listed as *S. pneumoniae*, the designation that is now generally accepted.

Although any of a large number of microorganisms may cause acute inflammation of the lungs (pneumonia),[110] "typical (primary) lobar pneumonia" is most commonly caused by the pneumococcus. Pneumococcal pneumonia is generally preceded by a simple acute viral infection of the upper respiratory tract such as the common cold and it may also occur as a complication of influenza. It has been called the "captain of the men of death" and "the friend of the aged," because it had long been the principal immediate cause and most frequent contributing cause of death in the aged and infirm and in those with otherwise fatal diseases.

Prior to the introduction of pneumococcal conjugate vaccine, the pneumococcus was also the most frequent cause of acute bacterial meningitis in those over age 3months (Finland, 1977).[239] This remains true today in countries where conjugate pneumococcal vaccine has not been introduced, being exceeded in frequency only should a sporadic meningococcal epidemic occur. In the pre-antibiotic era pleural empyema was one of the most common complications of pneumococcal infection and still remains a common and increasing problem.[52,264,120,83,159,51] In some studies the pneumococcus is the most frequent cause of empyema[111,83,159,51] occurring as a complication of either pneumonia or of otitis media at all ages.[168,30] In one pediatric study the incidence of pneumococcal empyema in children was found to be increasing despite the introduction of the pneumococcal conjugate vaccine.[51] In adults an increase in incidence of pneumococcal empyema may be occurring in association with concomitant HIV infection and in children exact reasons for the increase are not entirely apparent.[264,83,51] In both adults and children this increase appears to be occurring particularly in association with serotype 1 *S. pneumoniae*.[52,264,83,51] The importance of this in the pediatric population is that the current 7-valent pneumococcal conjugate vaccine does not protect against serotype 1, which may account for the findings of Byington and colleagues.[51] Less frequently, the pneumococcus may cause focal infections at other sites in the body. It is also the most common organism cultured from the blood of patients with acute febrile illness, usually pneumonia or meningitis, but not infrequently also from infants and young children with no demonstrable localized site of infection.[198,265,293]

2. Historical Background

The early history of the discovery of the pneumococcus and its pathogenic potential for animals and humans was presented chronologically and in some detail by

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The first edition of this chapter was written by the late Maxwell Finland and later versions by Robert S Baltimore, and Eugene D Shapiro. The current authors acknowledge the contributions of their predecessors.

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White,[287] subsequently, by Finland,[109] Austrian,[14,15] and, more recently, by Watson and Musher.[282] According to Austrian, the pneumococcus was probably first visualized in pulmonary tissues by Klebs in 1875, later by Eberth in 1880, and then by Koch in 1881. However, the organism was first isolated in the laboratory independently by Pasteur in France and Sternberg in the United States and reported by both in 1881; both these workers injected saliva subcutaneously in rabbits, who subsequently died and were shown to have large numbers of organisms in their blood. Pasteur used saliva of an infant that died of rabies; Sternberg injected his own saliva. Thus, both demonstrated the occurrence of the pneumococcus in the pharynges in carriers without relation to disease.

During the next few years, considerable controversy resulted from the descriptions and characterizations of the bacteria seen in pneumonic lungs by Friedlander, Weichselbaum, and Freinkel (quoted by Austrian[14,15]); this was eventually resolved by the application of Gram's stain described in 1884. The stain permitted the differentiation of Friedlander's bacillus, now known as *Klebsiella pneumoniae*, which was decolorized by Gram's method (gram-negative), from the pneumococcus, which was not decolorized (gram-positive).

During the late 1880s, reports were published of the presence of pneumococci in focal infections other than those of the lung, including valvular lesions in cases of endocarditis, purulent meningitis, otitis media, arthritis, sinusitis, conjunctivitis, and from other organs and sites. The syndrome of endocarditis, meningitis, and pneumonia known as Osler's triad has been termed Austrian's syndrome when it has a pneumococcal etiology.[12,81]

At the turn of the century, Neufeld[204] reported on the solubility of pneumococci in bile, which became a useful tool in the differentiation of pneumococci from other streptococci, especially the viridans variety. Most important were the demonstration of the development of antibodies against the homologous pneumococcus following infection or vaccination, which protected animals against subsequent infection with that organism, and the description of the specific agglutination and quellung reactions in homologous antisera by Neufeld[205] in 1902. The agglutination reaction led to the more definite segregation of pneumococci into specific types in 1913 by Lister[179] in South Africa and by Dochez and Gillespie[79] at the Rockefeller Institute in New York.

Some partially successful attempts were made to protect the highly susceptible workers in the gold and diamond mines in South Africa by vaccination with mixtures of these pneumococcal types.[180,215] This also led to the development of specific antisera for therapy and the use of the agglutination reaction to identify the causative type before administering the therapeutic antisera. It was not until 1931, however, that Armstrong[10] in England first reported the clinical use of the quellung reaction for the rapid typing of pneumococci. By 1932, Cooper et al.[66] in New York City had segregated more than 30 specific types; subsequently, workers in the United States and in Denmark increased that number to the 91 types known at present.[220,186,148] Most recently, the genetic sequences of the enzymes that build the capsules of all 91 types have been published.[24,219] The typing of pneumococci and the type-specific antibody response to infection or to immunization, first with whole organisms[292] and later with type-specific capsular polysaccharides,[163] proved to be potent tools that enhanced the capabilities for epidemiological studies of pneumococcal infections[237] and offered the possibility of control of infections in individuals and in susceptible population groups. Unfortunately the radio-immunoassay used to determine serotype-specific antibody responses[237] was first shown in the 1990s to be too non-specific; adsorbed ELISA assays are now the state of the art for measuring pneumococcal antibody.[285]

Meanwhile, progress was being made, notably at the Rockefeller Institute, in delineating the biology of the pneumococcus and its biochemical constituents and immunogenic properties. Most important was the demonstration that the type specificity, pathogenicity (virulence), and eventually also its immunogenicity resided in the specific chemical composition of its capsular polysaccharide. When purified, this component proved to be essentially nontoxic when injected in humans and capable of inducing type-specific antibodies in varying quantities. When it was also shown that 80% of all serious and life-threatening pneumococcal infections, bacteremia, meningitis, endocarditis, and infections of normally sterile body cavities were caused by only 14 of the known 80 or more types, a vaccine incorporating these 14 types was made available,[163] and early trials showed it to be effective in many circumstances. The 23-valent vaccine has been shown in indirect cohort studies to prevent invasive disease, but has not been shown in randomized trials to prevent non-bacteremic pneumonia in the elderly.[157] The great leap forward in the prevention of pneumococcal disease in the new millennium has been the development of conjugate pneumococcal vaccine, which prevents both invasive disease and non-bacteremic pneumonia in immunized children.[28,173,208,71] Through its impact on carriage, this vaccine also has been shown to reduce the burden of invasive pneumococcal disease due to the pediatric serotypes in adults.[288] The effects of the conjugate vaccine on the control of pneumococcal infections are a major subject of contemporary investigation of pneumococcal disease. A separate section of this chapter is devoted to the development

of pneumococcal vaccine and the benefits of its use. Since the late 1970s, there has been concern about the existence of pneumococci resistant to penicillin and other antibiotics.[158] In the 1980s, 1990s, and during the first decade of the 21st century rates of antibiotic resistance have been alarmingly high.

3. Methodology

3.1. Sources of Epidemiological Data

In introducing his chapter on the epidemiology of pneumonia, Heffron[145] indicated that the absolute frequency of occurrence is unknown. Statistics from various health organizations throughout the world provide information on the total number of deaths from all causes, including pneumonia, but in many instances, especially in Africa, where the disease is widely prevalent, deaths are frequently unreported and inaccurate. In the United States, pneumonia and influenza deaths from 121 cities are reported in the Centers for Disease Control and Prevention (CDC)'s *Morbidity and Mortality Weekly Report* (*MMWR*), but they are generally regarded as an index of influenza mortality. As shown in Section 5.15, the role of the pneumococcus in these infections may have been underestimated. In 1934, Collins and Gover[64] reported that acute infections of the respiratory tract caused more deaths and a higher morbidity in the United States than did any other group of diseases. However, it is impossible in the United States or elsewhere to obtain exact figures on morbidity or mortality because pneumonia has not been a reportable disease in most states; it is estimated that even in those states wherein it is reportable, only about half the cases are actually reported. Also, there is no uniform case definition of pneumonia. In temperate zones, lobar pneumonia, but not bronchopneumonia, has a definite seasonal incidence, but their differentiation is often impossible. In the United States, the best estimate of the incidence of pneumococcal pneumonia has come from special surveys (see Section 3.2). The global burden of pneumonia is estimated annually by WHO and it is considered to be the leading infectious cause of death in adults.[289] In children there is evidence that pneumonia causes an increasing proportion of deaths in the poorest countries with the highest rates of infant mortality.[290] Pneumococcal meningitis, while also not a reportable disease, has been included in special meningitis surveys.[56,154,284,239]

3.2. Surveys

A number of surveys have been carried out to estimate the incidence of pneumonia. These include a population study of 2,602,000 people conducted by the federal government for 1935–1963, data from the Cleveland family study reported in 1936, health records from the Kaiser-Permanente Health Center, and reports from hospitals such as the large retrospective regional study performed in Sweden from 1964 to 1980.[49] These have been summarized by Mufson,[200] Austrian,[14,15] and the CDC.[56]

A number of studies have focused on specific populations of interest. Children were the subject of nationwide prospective studies from Finland[87] and Israel.[72] In the United States, specific populations at exceptional risk for pneumococcal infections have been surveyed including inmates of a correctional facility,[151] Alaskan natives,[76] and Native Americans.[67] Data on these populations are presented in Table 1 and discussed in Section 5.1. Antibody surveys have not been reported except when associated with vaccine trials. The assays are not sufficiently specific for use in establishing estimates of incidence.

3.3. Laboratory Diagnosis

3.3.1. Isolation and Identification of the Organism. Growth of pneumococci requires suitable media such as nutrient broth to which serum or defibrinated blood such as that of rabbit, sheep, or horse is added. For epidemiological purposes, however, reliance has been placed on three methods: (1) direct culture on solid media (blood agar); (2) in the past by mouse inoculation;[236] and (3) application of the Neufeld quellung method, latex agglutination, or PCR of the capsular locus to cultures or to fresh materials (sputum, exudate, blood cultures) for specific typing of pneumococci. Agglutination of antibody-coated latex beads has replaced the quellung test in many laboratories for the serotyping of pneumococci, and can be done on CSF for the serotype-specific diagnosis of meningitis[248] and can be used for rapid identification of bacterial colonies in the laboratory by testing blood culture supernatents. Identification of common pneumococcal antigen in urine[234,259] may become a standard in pneumococcal clinical trials and its role in clinical practice is under evaluation. While the assay is specific, it lacks sensitivity and is not useful in children.[88] For cultures of materials from air, dust, skin, or fomites, broth-moistened swabs of the material or surfaces may be streaked directly on blood agar; characteristic colonies of pneumococci are easily recognized after incubation for 12–24 h (as are those of hemolytic or viridans streptococci and staphylococci). The swab may then be placed in blood broth and pneumococci identified from subcultures on blood agar. Initiation of growth of pneumococci may be enhanced by incubation in 5% carbon dioxide or in a candle jar. Additional cul-

Table 1. Focused Studies of the Incidence of Pneumococcal Disease[a]

Years of study	Population surveyed	Size of population surveyed	Incidence (per 100,000), unadjusted	Case-fatality rate (per 100)	Reference
1986–1990	Native Alaskans	81,368	74	13–14	35
1986–1990	Non-native Alaskans	438,763	16	13–14	35
1986–1990	Native Alaskans, less than 2 years of age	4,068	624	1–2	35
1983–1991	Native Americans, White River Indian Health Service Hospital, total unadjusted	9,814	207	5%	36
1983–1991	Native Americans, White River Indian Health Service Hospital, Children less than 2 years old	654	1,820 (2,396, 1–2 yrs old)	0%	36
1989 (4 weeks)	Correctional facility, USA	6,700	687		34
1988–1990	Israeli, children 0–12 years old	2,400,000 est	19.9	2.2	33
1988–1990	Israeli, children, less than 1 year old	NA	104	30 (first month of life); 2.1 (one month to 12 months of life)	33
1985–1989	Finland, children, 0–15 years old	5,000,000 est	8.9	1.3	32
1985–1989	Finland, children, less than 1 year old	NA	37	NA	32

[a]Standard of diagnosis, isolation of *Streptococcus pneumoniae* from usually sterile sites.
NA, data not available.

tures in broth, and in the past mouse inoculation, were used to detect multiple serotypes and to enhance the yield. Nasal or pharyngeal swabs may be plated directly on blood agar. Prior exposure to antibiotics (during prophylaxis or therapy) markedly reduces or may eliminate the chance of growing pneumococci from the nose and throat. Carriage studies often use direct plating on blood culture agar plus 5 μg/ml gentamicin to suppress growth of viridans streptococci, but the liquid STGG (skim milk, tryptone, glucose, glycerol) both medium more recently been usedto allow retention of the initial swab inoculum.[209]

3.3.2. Serological Methods. Serological tests for demonstrating type-specific antibodies have included opsonic or pneumococcidal tests in fresh defibrinated or heparinized whole blood, the mouse protection test on serum, macroscopic or microscopic agglutination of heat- or formalin-killed suspensions of organisms, or precipitin and diffusion tests with supernatant fluids of cultures or exudates or with type-specific polysaccharides. These tests differ in sensitivity and also somewhat in specificity. Specific antibody has been quantitated with specific polysaccharides and used in surveys for susceptibility in relation to immunization and to measure the response to conjugate and polysaccharide vaccines. The current gold standard for measuring pneumococcal antibody is enzyme-linked immunoassay (ELISA).[285]

4. Biological Characteristics of the Organism

Morphologically, the pneumococcus typically appears as an ovoid or spherical, coccoidlike form of 0.5–1.25 μm, in pairs, the distal ends of which tend to be pointed or lancet-shaped and surrounded by a distinguishable capsule that contains polysaccharide. The diplococci may occur in chains, particularly in liquid medium, hence the designation streptococcus. The chemical composition of the polysaccharide determines its specific serological type, of which there are now at least 91,[148,220] including several that are closely related serologically, such as serotypes 6A, 6B and 6C. The diplococci usually stain gram-positive, but may decolorize and appear gram-negative in old cultures or after exposure to antibacterial agents. When grown on blood agar, pneumococci form clear, round, mucoid, colorless, umbilicated colonies surrounded by an area of green hemolysis. Heavily capsulated organisms, such as those of type 3, grow in dome-shaped, clear, mucoid colonies.

Resistance of pneumococci to chemotherapeutic agents was recognized as emerging during therapy with ethylhydrocupreine (optochin) in 1918, the first chemical to receive extensive clinical trials in pneumonia.[199] Resistance to sulfapyridine was also recognized as a possible cause of failure of that drug in the treatment of pneumococcal pneumonia in 1938.[286,185] Although significant resistance of pneumococci

to penicillin had been infrequent and limited in geographic distribution in the 1970s and early 1980s, in the past 20 years this has become a serious and increasing worldwide problem (see Section 5.1.1). Resistance to other antibacterial agents has been reported from many areas and has accounted for therapeutic failures. Reports have included resistance to sulfonamides, tetracycline, erythromycin, lincomycin, chloramphenicol, β-lactam antibiotics,[169,7] and more recently the fluoroquinolones.[59] Pneumococci are also relatively resistant to aminoglycosides and some are highly resistant to streptomycin. Multiantibiotic-resistant pneumococci have now been reported globally (see Section 5.1.1).

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

In the United States, a federal government survey over a 12-month period in 1935 that involved over 2.6 million people in 83 widely scattered cities and 24 rural counties suggested an incidence rate of 558 cases of pneumonia per 100,000.[14,15] Data from several centers reported for 1920–1928 indicated that deaths from pneumonia generally accounted for 7.4–8.3% of deaths from all causes. On the basis of data from various sources, Austrian[14,15] estimated ("with limited confidence") that the attack rate of pneumococcal pneumonia in the United States lay somewhere in the range of 1.5–10 (more likely between 2 and 5)/1,000 man years of exposure.

The Cleveland study of illness in middle-class families comprising 292 adults and their school-aged children, reported in 1953, has been summarized by Austrian.[14,15] There were three illnesses diagnosed as pneumococcal over a 3-year period, 1974–1976, with an attack rate of 10.3/1,000 man years. At the Kaiser-Permanente Health Center, the estimated attack rate of pneumococcal pneumonia of all types was 1.4/1,000/yearamong Californians 45 years of age or older. However, among institutionalized patients, the attack rate was calculated to be 12.5/1,000/year forpneumonia due to all pneumococcal types.[14,15]

Data on the incidence of pneumococcal infections has come from the CDC[56] and from a detailed review of morbidity and mortality by Mufson.[200] In the early 1980s, the CDC estimated rates per 100,000/year of pneumococcal pneumoniaof 68–260, of pneumococcal meningitis of 1.2–2.8, and of pneumococcal bacteremia of 7–25; the case-fatality rates of these syndromes have been 5–7%, 32%, and 20%, respectively.[56] Mufson's estimates of incidence, converted to rates per 100,000, were 100–200 for pneumonia and 0.3–4.9 for pneumococcal meningitis based on published studies from the mid-1970s. Higher rates of pneumococcal infection were observed in patients with sickle-cell anemia, congenital asplenia, renal transplant, Hodgkin's disease, and multiple myeloma than in subjects without these preexisting conditions.[200]

The importance of *S. pneumoniae* in different clinical syndromes at different ages has been reviewed by Klein[168] in infants and children and by Mufson[200] in the general population. On the basis of recent cooperative studies, the pneumococcus was found to be the most common pathogen cultured from the respiratory tract in infants (other than neonates) and children, and it is also the most frequent cause of serious infections, including pneumonia and bacteremia.[168]

In the general population of nine cities, *S. pneumoniae* was the most common cause of community-acquired pneumonia requiring hospitalization: pneumococcal pneumonia accounted for 26–78% of all pneumonia cases in the nine areas and in most studies exceeded 50%.[200] Mortality rate varied from 6 to 19%, but was two to five times higher in the 20% of adults who developed bacteremia. Indeed, this organism is the most common cause of bacteremia in adults, as well as in children over 1 month old.[200] As shown in Table 1, children and residents of closed communities, such as prisoners and infants, all have a risk of invasive pneumococcal infection far above the estimates for normal populations. While populations can be found with an annual rate of 20 or less per 100,000, children in the first 2 years of life and certain populations such as Native Americans have a much higher rate of illness. In the Israeli study by Dagan et al.,[72] it was shown that within Israel the rate of pneumococcal illness was much higher among the Arab population than among Jews, and the case-fatality rate was 5.7% among non-Jews and 0.3% among Jews.

Pneumococcal meningitis occurs at a rate of about 0.3–4.9 persons/100,000, with children under age 5 years at higher risk.[200] In a summary from the CDC reviewing reports of meningitis from 38 states in 1978, the incidence per 100,000 of *S. pneumoniae* meningitis was 0.30.[55] It was exceeded by *Haemophilus influenzae* meningitis and *Neisseria meningitidis* meningitis, with rates per 100,000 of 1.24 and 0.72, respectively (see Chapters 16 and 23).The incidence of pneumococcal meningitis was highest in the first year of life (age-specific rate of 8.0/100,000). While the absolute incidence is much less in older age groups (0.1–0.4/100,000), its *relative* importance increases, and this organism is the most common cause of meningitis

for elderly individuals in the United States. In a CDC-sponsored study of six states surveying 34 million people in 1986, the incidence of pneumococcal meningitis was 1.1 per 100,000 compared with rates of 2.9 and 0.9 per 100,000 for *H. influenzae* and *N. meningitidis*, respectively.[284] With the dramatic decline of *H. influenzae* meningitis in the early 1990s due to the success of the conjugate vaccines, prior to the introduction of conjugate pneumococcal vaccine *S. pneumoniae* was the most common cause of bacterial meningitis.[239] In a study of children from Israel, 1988–1990, the rate of pneumococcal meningitis was 5.4/100,000 for children less than 5 years of age, but in infants less than 1 year of age it was 16.7/100,000.[72] In Finland, 1985–1989, the rate of pneumococcal meningitis was 2.1 per 100,000 for those under 5 years of age and 6.8/100,000 for those under 1 year of age.[87] *S. pneumoniae* is also the most common aerobic organism grown from blood of febrile infants and children in whom no focus of infection is determined. The highest rate of pneumococcal carriage in the United States is also among preschool children; it is somewhat lower in older children and is about the same in adolescents as in adults, but in the last two groups, carriers are more frequent in households with preschool children.

The commonest pneumococcal infection is pneumococcal otitis media of which there are thought to be approximately 7 million infections per year in the United States alone, with more than 60% of infants experiencing at least one episode of otitis media during the first year of life (Teele, Lein et al., 1989).

5.1.1. Occurrence of Penicillin-Resistant Pneumococci. Penicillin has historically been the antibiotic of choice for most cases of pneumococcal infection. There has been monitoring of pneumococci worldwide for susceptibility to this agent, and in the past, growth of pneumococci has generally been inhibited by a concentration of less than 0.05 μg/ml of penicillin. Relatively resistant strains (now referred to as intermediately resistant) had been reported with low-frequency prevalence worldwide since 1967 when a well-documented case of respiratory infection due to a pneumococcal strain with an MIC of 0.6 μg/ml of penicillin G was reported from Sydney, Australia.[138] The patient involved was a 25-year-old with hypogammaglobulinemia and bronchiectasis who had received many courses of antibiotic treatment in the past. Since then there have been many reports of such isolates. Until the 1980s, there were only sporadic reports of strains intermediately resistant, with MICs of 0.1–1.0 μg/ml to penicillin. Strains resistant to 2.0 μg/ml of penicillin are termed high-level resistant strains using meningitis breakpoints. The Clinical Laboratoy Standards Institute (CLSI) has recently published new breakpoints for pneumonia of intermediate susceptibility at 4 μg/ml and resistance at 8 μgml for treatment with parenteral penicillin.

Beginning in South Africa in 1977,[8,158] there have been reports of pneumococcal isolates resistant to penicillin, using meningitis breakpoints with MICs at 2–16 μg/ml. These strains generally had resistance to multiple antibiotics including tetracyclines, streptomycin, chloramphenicol, erythromycin, clindamycin, and sulfonamides. Only a small number of pneumococcal serotypes have been associated with high-level resistance. Generally, the level of resistance to penicillin correlates with resistance to all semisynthetic penicillins and cephalosporins.[7] Resistance of pneumococci has not been associated with the production of β-lactamases but with the alteration in the cell-membrane-associated enzymes that are responsible for cell wall assembly and that bind penicillin. Alteration of these penicillin-binding proteins has been associated with high-level resistance[295] as well as low-level resistance,[137] and penicillin tolerance.[53,271] (Penicillin tolerance refers to strains requiring a substantially higher concentration of penicillin to kill them than to inhibit their growth.) Strains of pneumococci can develop increasing resistance in a stepwise fashion,[53] and the genes for altered penicillin-binding proteins may come from heterologous organisms as well as pneumococci.[271] It appears that resistant clones have spread throughout the world, although the rates of resistance vary considerably in different countries. Global-resistant clones are now defined by the Pneumococcal Molecular Epidemiology Network.[196]

The outbreak of multiply-resistant pneumococci that began in Johannesburg, South Africa, in 1977, deserves special mention. The first isolate was a serotype 19A from the sputum of a hospitalized 3-year-old with pneumonia. Additionalisolates were recovered by screening patients and hospital personnel from the same hospital using an agar-disk diffusion method for screening pneumococcal respiratory isolates. It was clear that these were virulent strains as there was considerable morbidity and some deaths attributable to invasive infection due to these pneumococci. These isolates were resistant to penicillin concentrations of 0.12–4 μg/ml and were found in 29% of 543 pediatric patients and 2% of 436 hospital staff members.[158] The year 1977 appeared to be a watershed year as resistant pneumococcal strains were reported from Durban, South Africa; Minneapolis, Minnesota; and London, England.[158] The report from Durban involved five cases of meningitis or sepsis in children from 3 months to 2 years of age. Each had severe malnutrition, with recent courses of antibiotic treatment. Three cases occurred while the children were hospitalized and receiving antibiotics. The isolates were serotype 19A and had MICs of 4–8 μg/ml of penicillin and exhibited resistance

to other β-lactams, aminoglycosides, and chloramphenicol. The three children with meningitis died and the two with septicemia responded to treatment with alternative antibiotics.[8]

Intermediately susceptible strains (penicillin MICs 0.1–1 μg/ml) are clearly less susceptible than are usual strains, but in the absence of meningitis, infections due to these strains are often successfully treated with high doses of penicillin.[53,217] A prospective study of 504 patients in Spain demonstrated a similar outcome in patients treated with penicillin or cephalosporins whether their pneumococcal isolate was fully susceptible to penicillin or less susceptible.[217] There have been a number of recent reviews which conclude that intravenous penicillin and highly active intravenous cephalosporins such as cefotaxime and ceftriaxoneare adequate therapy for intermediately resistant pneumococcal pneumonia.[172,226] These data support the higher breakpoint (4 μg/ml) for clinically relevant resistant infections outside of the CNS. High doses of intravenous penicillin or ceftriaxone provide adequate drug concentrations above an MIC of 4 μg/ml for at least 40–50% of the dosing interval (ceftriaxone given twice daily). Macrolide-resistant strains do not, however, respond to macrolide therapy[183](Daneman, McGeer et al., 2006), nor do fluoroquinolone-resistant strains respond to treatment with those agents.[77]

The cause of the increase of antibiotic resistance is multifactorial. There is abundant evidence that prior exposure to antibiotics is a risk factor for infection due to resistant strains and may play an important role in rising rates of resistance.[9,36,279] The exceptional risk of children and the high prevalence of "pediatric" serotypes among resistant strains (serotypes 6, 14, 19, 23) provide evidence that indiscriminate use of antibiotics for minor pediatric infections may be a major factor driving this epidemic.

5.1.2. Distribution of Pneumococcal Types. The importance of pneumococcal types and their distribution in various populations need to be stressed. In the past, when immunotherapy with type-specific serum was used, typing of organisms and provision of the proper sera required knowledge of the prevalent serotypes. Since the introduction of the pneumococcal polysaccharide and conjugate vaccines, which induce type-specific immunity, it is now necessary to know the prevalence of pneumococcal serotypes in order to prepare an appropriate multivalent vaccine. Because the frequency of the different serotypes varies with time and with different clinical syndromes,[112,115] constant monitoring continues to be a necessity for inclusion of the appropriate types in vaccine formulations.[21] There has been a fundamental shift in the serotype distribution of pneumococci causing invasive disease in adults in the United States with an increasing prevalence of pediatric serotypes (mainly 6A, 6B, 9V, 14, 19A, 19F, 23F) and a great reduction in disease due to the classic types 1, 2, and 5.[92] The distribution of pneumococcal serotypes causing invasive disease in children has been recently reviewed.[140,141,92,142] It is clear that immunocompromised patients[118] and patients infected with HIV[68,102,118,48] have an increased proportion of pediatric serotypes and these pediatric strains increase in frequency in adults > 65 years of age.[93]

Pneumococci have been classically serotyped according to the presence of specific carbohydrate antigens that make up the capsule of the bacterium. Rabbit antisera specific for each serotype are used. There have been two serotype designation schemes used, the Danish and American systems.The Danish system is now dominant and classifies strains with a number designation and sometimes a letter following the number. A serogroup number represents a group of antigenically related strains. Each type within the group is indicated by a suffix letter (e.g., serogroup 6 contains serotypes 6A, 6B, and 6C). The propensity of a given serotype to cause invasive disease varies greatly and a ratio of invasiveness has been established.[43] Multiple types of pneumococci can be isolated from infected patients and carriers at the same time or at different times and may persist for varying lengths of time, continuously or intermittently in carriers as well as after recovery from pneumonia. The role of these types of infections in the individual patient may be surmised from the clinical findings of the demonstration of antibodies to the homologous types during the infection or convalescence. The serotypes present in the current vaccines and those most commonly encountered in bacteremic pneumococcal diseases of adults and children are summarized in Tables 2 and 3

Table 2. Serotypes Most Commonly Associated with Bacteremic Pneumococcal Infections in Children and Adults (Danish Serotype Designation)

Adults	Children
1	1
3	3
4	4
	5
7F	7F
6A,6B	6A,6B
8	
9N,9V	9N,9V
12	
14	14
19A,19F	19A,19F
23F	23F

Table 3. Antigenic Serotypes of Pneumococcal Polysaccharides Contained in Pneumococcal Vaccine

7-valent conjugate vaccine	23-valent vaccine
Danish system	Danish system
	1
	2
	3
4	4
	5
6B	6B
	7F
	8
	9N
9V	9V
	10A
	11A
	12F
14	14
	15B
	17F
18C	18C
	19A
19F	19F
	20
	22F
23F	23F
	33F

The 10 valent conjugate adds types 1, 5 and 7, to those in the 7 valent vaccine, while the 13 valent adds in addition to those in the 10 valent, types 3, 6A and 19A

from several sources. These change with time and place, so that individual reports may vary from the data shown. In the United States and most Western countries the most common serotypes encountered in adults are 3, 4, 7, 8, and 14[14,15]while in children the most common serogroups/types are 6, 9, 14, 19, and 23. Types 1 and 5 continue to be reported frequently in developing countries.[140,141] Tables 2 and 3 demonstrate that (1) the 23V vaccineincludes the types most commonly responsible for bacteremic disease in children and adults; (2) there is broad overlap in the serotypes seen in children and adults but some differences exist; and (3) bacteremic diseases in immunocompetent adults are largely due to serotypes with lower numbers; the higher-numbered serotypes are more commonly found in the pharynges of healthy carriers. In the era of conjugate pneumococcal vaccine, the pediatric serotypes are disappearing among both children and adults with replacement disease occurring in a number of serotypes, particularly type 19A.[22]

5.1.3. Occurrence in Carriers. Pneumococci are present in the nose or throat or both of about one fourth of all healthy persons at any given time; they are more prevalent in the winter months, and nearly all individuals carry them transiently or intermittently in the course of a year. In very young (<2 months of age) infants, they are usually found only if they occur in the mother, but in older infants, they are more frequent.[209] Mothers who smoke have increased pneumococcal carriage as do their infants.[136] The frequency of colonization in the very young is correlated with crowded poor socioeconomic living conditions such that 50% of children in aboriginal communities are colonized by 6 weeks of age[176] while it takes 6 months for a similar level of colonization to occur in US children.[134]

The presence of upper-respiratory infections is not ordinarily a determinant in the individual, but epidemics of respiratory infections tend to increase the spread of disease-producing types of pneumococci, particularly in families of cases.[75]

The epidemiology of pneumococcal infections was discussed in some detail at a symposium on the *Pneumococcus*[228] in 1980 in which Riley and Douglas from Papua New Guinea likened the situation in their country to that in the New World at the turn of the century.[231] Acquisition of pneumococci in the nasopharynx begins in infancy, reaches the highest rates in preschool children, and then declines in advancing age groups. The rates in New Guinea, as elsewhere, are generally related to the amount, degree, and duration of contact with cases or other carriers.

5.2. Epidemic Behavior and Contagiousness

As early as 1888, Netter[203] presented evidence, mostly on clinical grounds, for the contagiousness of pneumonia and for the part played by the pneumococcus. He described family outbreaks of pneumonia and other pneumococcal infections even in the same household. He and others[69,161] traced cases to direct or close contact with other cases or convalescents. He found that pneumococci survived in dried sputum for many months and he recovered the organisms from the dust of hospital rooms.

Epidemics involving multiple cases in families[114,42] or in institutions[251,252] were described in which direct contagion was traced either separately or during outbreaks in small communities. In some of them, the pneumococcus was identified by culture or mouse inoculation.[273] Outbreaks of focal pneumococcal infections have been recorded both in association with pneumonia and independently. Pneumonia acquired in foundling homes was recognized early on as an important cause of high death rates in infant asylums and as a frequent cause of deaths in babies who were healthy on arrival. Direct contagion was also recognized as the cause of the spread of pneumonia among workers in mining camps in South Africa,[132,215,213,214] as well as during the construction of the Panama Canal[145] in which overcrowding, especially

in sleeping quarters, and high attack rates soon after arrival were the two most important features.

Pneumonia was the leading cause of death in army camps during the Anglo - Boer War of 1899 – 1902 and World War I,[273,63,250,218] and occurred mostly in relation to epidemics of measles and influenza associated also with hemolytic streptococci, but the majority of cases and deaths after and even during such epidemics were typical pneumococcal lobar pneumonia. In 1919, epidemics of type 1 pneumococcal pneumonia were reported from Camp Jackson[218] and Camp Upton[266] and of type 2 from Camp Grant.[149] New draftees and black recruits were the most susceptible. Direct contagion was later demonstrated when typing was used. In 1940, spread of pneumococcal pneumonia could also be traced in some hospitals from bed to bed.[152] In 1937, Harris and Ingraham[139] described an epidemic of type 2 pneumonia in the Civilian Conservation Corps confirmed in many cases by serological studies; these involved primarily members of the corps in the same barracks and only two officers who had the most contact with the men. Reports have stressed smaller epidemics and nosocomial spread of resistant strains;[8,158,247] the association of pneumococcal disease with influenza;[200] and conditions responsible for greater susceptibility to pneumococcal infection (see Section 7.1). An outbreak of pneumococcal pneumonia in a jail reinforced the importance of overcrowding and poor ventilation as contributing factors.[151] Epidemics of meningitis separate from pneumonia have recently been described in Africa, although the pneumonia burden was not quantified.[177]

5.3. Geographic Distribution

As already noted, reliable information on the geographic distribution, prevalence, and incidence of pneumonia and particularly of other pneumococcal infections is unknown because of lack of adequate reporting in most parts of the world and the unreliability of those reports that are available. It may be said, however, that such infections are ubiquitous worldwide, and endemic and, under some conditions, may occur in well-circumscribed epidemics. Although usually thought to be particularly prevalent in temperate zones, they also occur in warmer regions and in the tropics.[145] Temperature and humidity appear to influence the prevalence of pneumonia, and in countries where the most abrupt and severe changes occur in winter, the incidence of the disease is correspondingly high during such times.[135,233]

Among admissions to the Boston City Hospital, the incidence of pneumococcal bacteremia[197] and meningitis (Finland 1977) decreased after 1935 and again after 1941 with the general use of sulfonamides and penicillin, but it fluctuated within a moderate range after that. The case-fatality rate after 1941, however, still remained high in cases of meningitis and varied with age in other bacteremic cases, being highest in infants and in persons 70 years or older.

5.4. Temporal Distribution

Although the low numbered serotypes dominate reports of invasive disease in both children and in adults, during the early part of the 20th century, over the course of the century the higher numbered pediatric serotypes became more common reflecting perhaps a shift from direct contagion, to disease caused by circulating carriage types in the very young and the very old, as well as in immunocompromised persons in middle age.[93]

5.5. Age

Pneumococcal infections occur at all ages, although they occur more commonly at extremes of age (Jokinen, 1993). They are most frequent in the first year of life, decrease rapidly to a low between 10 and 15 years, and then increase in incidence steadily in advancing age groups. Deaths from such infections are also most frequent in the first year of life, least frequent between 15 and 20 years, and rise with advancing age groups more steeply than do the case rates.[145] A change has been noted in the age of adult patients with invasive pneumococcal infections in association with the HIV epidemic. HIV-seropositive patients with invasive pneumococcal infections, including pneumonia, are usually found to be younger than HIV-seronegative cases.[162,101] The estimates of incidence for invasive pneumococcal infection in Goteborg, Sweden, which are typical of a developed country, are 25.8/100,000 in the first year of life, falling to 2/100,000 in the second decade, then rising by decade and peaking in the eighth decade at 16.4/100,000.[49] The case-fatality rate from pneumonia and meningitis increases with each decade, from under 10% in the young to over 50% in the eighth decade. The age distribution of cases of pneumococcal meningitis followed the same trend among patients at Boston City Hospital during 12 selected years between 1935 and 1972, but the case-fatality rates were higher, about 60%, for those in the first decade of life, 50% in the second decade, and increased with each decade thereafter to 81% in patients 70 years old or older (Finland, 1977). It is not just increasing chronological age that predisposes to pneumococcal disease, but also the increasing frequency of the underlying comorbid conditions associated with older age.[94]

5.6. Gender

Among cases of lobar pneumonia occurring in Massachusetts between 1921 and 1930, males predominated over females (58/42) in all age groups, but male predominance was less among infants and young children.[145] Among bacteremic patients at Boston City Hospital in 1972, the ratio was 51.7% males and 48.3% females for all patients.[197] The ratio of males to females among cases of pneumococcal meningitis for the 12 selected years between 1935 and 1972 (Finland 1977) was much higher and averaged 65% male for all ages, but varied in different age groups: 67% males among infants (<1 year old), 78% males in those 1–9 years old, 100% males among the small number of 10–19 years, and dropping steadily with each decade to 50% males among patients 70 years or older. Concomitant with the HIV epidemic changes have been noted in the gender ratio. Studies comparing HIV-seropositive and HIV-seronegative cases with bacteremic pneumococcal pneumonia have noted a greater number of female patients among the HIV-seropositive patients.[101,48]

5.7. Race

It has long been recognized that black people are peculiarly susceptible to pneumonia when they reside in cold climates, especially when moved from warm tropical regions. Under certain conditions, there is also a pronounced susceptibility in black people to pneumonia and pneumococcal infections in general even in warm climates. For example, high rates were encountered among the construction workers, who were predominantly black, during the building of the Panama Canal and among pneumonia patients elsewhere in Central America and tropical Africa.[145] Death rates from lobar pneumonia in various widely scattered states in the United States are also considerably higher in black people, sometimes twice as high as among white people. Thus, while true genetic susceptibility has not been separated from socioeconomic factors, black people are most likely to be subject to crowding in occupational as well as residential life (which is related to risk of infections), and may have a higher incidence of certain lifestyle risks[200,151] and reduced availability of medical care. Rates of invasive pneumococcal disease are also higher in indigenous communities such as Alaskan communities.[147]

5.8. HIV Infection

HIV infection itself is a significant risk factor for pneumococcal pneumonia.[91] While the exact mechanism(s) predisposing to pneumococcal infections are uncertain a number of studies have investigated possible reasons.[242,274,202,82] Impaired natural immunity to pneumolysin may be an important factor predisposing to bacteremic pneumonia.[3] Impaired opsonic activity of type-specific IgG in HIV-1 infected Ugandan adults appears to predispose these individuals to invasive pneumococcal infections.[262] Many risk factors for pneumonia in HIV-seronegative patients, such as comorbid illness, cigarette smoking, previous pneumonia, alcoholism, cirrhosis, neutropenia, low serum albumin, also predispose HIV-seropositive patients to pneumonia.[242,274,202,82] Conversely, factors associated with a lower risk for pneumonia in HIV-seropositive patients include pneumococcal vaccination, co-trimoxazole prophylaxis, and the introduction of highly active antiretroviral therapy.[91]

5.9. Cigarette Smoking

It has been known for a long while that cigarette smoking predisposes patients to community-acquired pneumonia.[2,206,207] Cigarette smoking has a number of detrimental effects on both immune and non-immune defence mechanisms in the human. Abnormalities induced by cigarette smoking that may predispose individuals to pneumonia include impaired mucociliary function, effects on immunoglobulin production and function, macrophage function, maGophge function, and effects on macrophage, polymorphonuclear leukocyte, and lymphocyte function. There is a specific risk for pneumococcal pneumonia in smokers[206,207] and cigarette smoking appears to be an independent risk factor for invasive pneumococcal infections. These investigators documented that the risk of pneumonia showed a positive trend with increased duration of smoking, increased number of cigarettes smoked, and the cumulative consumption of cigarettes.[206,207] Similarly HIV-seropositive patients who smoke cigarettes and/or drugs are also at increased risk of pneumonia.[98]

5.10. Occupation

Pneumonia has been known to be unusually prevalent in certain mining and milling industries.[44] In some mills, this has been associated with a high carrier rate that has been followed by an outbreak of pneumonia in the community.[129] In certain mining regions, the living conditions of the workers themselves, rather than the circumstances surrounding the work, may be the determining factor in spread of the disease. This was true in a group of steel workers who were crowded into small shanties in 1917,[261] among workers and their families during the construction of the Panama Canal in

1913,[132] among miners in South Africa,[211,215,182,212] and among plantation workers.[84]

The high attack rate in South African miners may occur because of the social environment of the gold mines. The community regularly receives non-immune recruits who have lived in remote rural areas and not been previously exposed to invasive pneumococcal serotypes/groups. The organisms spread rapidly through the closed communities and these recruits are at increased risk of pneumonia for approximately 6 months after recruitment.[128] This has sometimes been referred to as "recruit" disease.

5.11. Occurrence in Families

Today little attention is paid to the spread of pneumococcus within families, and the occurrence of a single case of invasive disease in a family does not merit the attention and use of prophylactic antibiotics that cases of meningococcal or *H. influenzae* disease do. Nevertheless, since prolonged intimate contact is required for spread of the pneumococcus, it is not surprising that spread in families does occur. Heffron[145]mentions a number of reports of multiple family members being infected with the same serotype of pneumococcus. In some cases affected family members were carriers, but in many there was an outbreak of invasive infections. The occurrence and spread of pneumococcal infections among members of the households of patients admitted to Boston City Hospital with lobar pneumonia were reported by Finland and Tilghman[114]and by Brown and Finland.[42] They included multiple cases of infections with the same type as the index case; some had lobar pneumonia and others had empyema, primary pneumococcal meningitis, otitis media, simple upper-respiratory infections, or bronchopneumonia, or were carriers without evidence of infection. Some cases admitted with lobar pneumonia were traced to contacts with other cases already in the hospital, but not from the same household. Serological studies demonstrated the presence or development of antibodies to the same types. The appearance and spread of additional new types among members of the household were also noted; these were also associated with pneumonia or focal infections or mild upper-respiratory infection and with the development of specific antibodies to the homologous types in a large percentage of members of these households. The advent of molecular typing allows the study of pneumococcal spread within families at the level of the clone within serotype. Spread of clones within families has been addressed in two recent studies. In the first, the index case was a child in day care and little spread of strains was demonstrated from children to their parents, suggesting a biological barrier in transmission between children and healthy adults.[230] On the other hand, a study from Japan showed that when the index case is an adult susceptible to pneumococcal disease (i.e., an infected adult), the pneumococcal strain causing that infection can often be found circulating among the children in the family.[153]

5.12. Occurrence in Animals

The occurrence of pneumococcal carriers and of widespread epidemics of pneumococcal infections among animals was reviewed by Finland.[109] They probably play very little or no part in infections and outbreaks in humans beyond causing occasional cases among animal handlers and will not be considered here.

5.13. Laboratory Infections

Cases of pneumonia acquired in the laboratory are not frequent. They have been associated with exposure to aerosols[232] or droplets or with handling of sputum.[23] They are of interest in delineating the incubation periods of pneumonia in man, since the clinical evidence of infection was manifest from 36 h to 3 days after a single exposure. Various types, including 1, 2, 7, and 18, were involved, and the same individual has been subject to more than one attack, but with different types and at different times.

5.14. Nutrition

The effect of specific nutritional deficiencies in predisposing to pneumonia is not known. Undernutrition, particularly of calories and proteins, is recognized as predisposing to infectious diseasesin general (see Chapter 1). There appears to be a greater risk of pneumonia in patients who are malnourished or have a low body mass index. Deficiencies and abnormality in cellular and circulating immune factors (e.g., complement) may play a role.[277] However, in contrast, one study undertaken in the United States investigating the relationship of lifestyle factors to the occurrence of community-acquired pneumonia noted a direct relationship between body mass index and the occurrence of pneumonia in females.[20] In both men and women in that study, an increase in weight of 40 lbs or more($\geq$ 18 kg) was associated with a twofold increase in the risk of pneumonia compared with individuals who maintained their weight.

5.15. Alcoholism

Alcoholism has been regarded as a major predisposing factor for invasive pneumococcal infections. In a Swedish study it was the most important risk factor in adults.[49] In

a US study, alcoholics also had a higher fatality rate from pneumococcal sepsis than did nonalcoholics, but only when accompanied by leukopenia.[225] The mechanisms of susceptibility of alcoholics to pneumonia are numerous and include factors such as enhanced oropharyngeal colonization, impaired mucociliary clearance, increased aspiration risk, defective surfactant production, decreased pulmonary function, macrophaye functionand effects on both humoral and cellular immunity.[95,96]

5.16. Association with Viral Infections

There are abundant lines of epidemiological, biological, and animal experimental evidence that pneumococcal infections occur more commonly and are of increased severity when they occur together with viral respiratory infections, including influenza.[224] The cough and mucus secretions associated with these infections may be important enhancers of pneumococcal spread in communities.[45] Recently it has been possible to determine the frequency of pneumococcal co-infection with viral respiratory pathogens by using conjugate vaccine (see Section 9.3 below) as a probe. These studies have documented that more than 40% of influenza hospitalizations for pneumonia in children,[188] similar percentages of RSV and parainfluenza infections,[188] and more than 50% of human metapneumovirus infections[191] leading to emergency room visits and hospitalization, are complicated by conjugate vaccine-preventable pneumococcal infection.

6. Mechanisms and Routes of Transmission

The most common route of transmission of pneumococci is by droplets from cases and carriers. In hospitals, the most frequent spread is from one patient to the patient in the adjacent bed, the disease rarely skipping a distance of more than two beds. Physicians, nurses, attendants, and others with frequent and close contact with patients are subject to colonization or infection from the patients unless they take special precautions to avoid or reduce exposure to the patients' droplets (coughing and sneezing), such as by masking the patient or themselves or both. Although pneumococci may becultured from the air or from the dust on the floors or in the rooms of patients with pneumonia they remain viable in dried secretions for long periods, probably as long as many days if not in direct sunlight.[287,145] Documented infections from such sources are rare.

In cases of pneumococcal meningitis following skull fractures, the infecting organism comes from the patient's own nasopharynx or accessory sinuses, and these foci are probably the sources of "primary" pneumococcal meningitis. The route by which the organism reaches the meninges in "primary" cases can often be traced to previous head trauma involving small fractures in the cribriform plate which may be difficult to recognize on X-ray. Suchfractures may be associated with recurrent pneumococcal (and other bacterial) meningitis that no longer recurs after the defect in the cribriform plate has been repaired. As the route of infection is directly from the nasopharynx, it is possible that serotype replacement in the conjugate vaccine era may be associated with more evidence of replacement in meningitis than in bacteremia, which is associated with a more restricted range of pneumococcal serotypes.

Otitis media in the acute form, and probably also acute sinusitis, usually occurs during or after simple upper-respiratory tract infections and is caused by the same type of pneumococcus found in the nose and/or pharynx or both. Experimentally, inflammation associated with occlusion of the eustachian tube (produced by influenza virus) is required to produce otitis media in the chinchilla after colonization of the nasopharynx with pneumococcus.[123,121]

7. Pathogenesis and Immunity

7.1. Pathogenesis

The incubation period for pneumococcal pneumonia is not known. Most sources state that it is 1–3 days. Heffron[145]suggests that it is "in the neighborhood of a few hours to about 48 hours". This would be for an infection from an exogenous source. Endogenous infection in a person who was previously an asymptomatic carrier and then developed invasive disease can occur with an "incubation period" of weeks, but in many cases infection as suggested above may follow a relatively short time after an upper-respiratory tract viral infection.

While significant details of human immunity to the pneumococcus are known, the exact mechanisms by which it multiplies in tissues and produce local inflammation or invasive disease remain to be fully elucidated. Pneumococci attach to respiratory epithelial cells by interaction with cell surface receptors. Mouse, rabbit, rat, and dog, and probably also humans, can remove large numbers of inhaled virulent pneumococci. This is done in part by the ciliated epithelium of the trachea and bronchi but in greater measure by the phagocytic cells, predominantly the pulmonary macrophage system, and is effected with or without the mediation of humoral antibodies.[164] It is known that damage to the ciliary system by alcohol or other inhaled toxic substances such as cigarette smoke or by minor viral respiratory

infections is necessary to establish pulmonary infection by pneumococci. In experimental animals, intrabronchial inoculation of pneumococci in a gelatin or starch plug is necessary to establish pneumonia, which extends out from the local alveoli to other parts of the lung.[65] In the dog, infection occurs in the local alveoli, and the pneumococci appear very rapidly in the local lymphatics, then in the hilar lymph nodes, then in the thoracic duct and subclavian vein, and finally in the peripheral blood.[80,267] The healthy mouse, in which a single virulent (e.g., type 1) pneumococcus injected intraperitoneally produces a lethal infection, can inhale a large number of the same organism from an aerosolized culture without ill effect; but the same aerosol will produce bronchopneumonia, bacteremia, and death if inhaled after the mouse has been anesthetized. Intranasal instillation of the broth culture will also produce pneumonia and death in the mouse[258] and in the rabbit.[257] A dermal pneumococcal infection has been produced by intradermal inoculation in rabbits; this lesion can be prevented by previous immunization and terminated by specific antiserum.[131] The same is true of experimental infections in the mouse and the dog. The importance of inflammation and obstruction of the eustachian tube for the establishment of pneumococcal otitis media was mentioned in Section 6.

7.1.1. Virulence Factors. The pathogenesis of pneumococcal infections is complex and involves the interaction of multiple possible virulence determinants with each other and with the immune system of the host.[61] These have been extensively reviewed elsewhere together with the biology of the pneumococcus and the molecular basis of pneumococcal disease.[70,272,184]

7.1.1.1. Capsule. The pneumococcal-specific capsular polysaccharides are complex polysaccharides, and the chemical structures of many have been elucidated in detail. These polysaccharides are responsible for the type specificity of protective antibodies that are elicited during infection[113] and the serological classification, which can be determined by the quellung reaction. The presence of the capsule is required for a strain to be pathogenic, and a larger amount of capsule production is associated with increased pathogenic potential. As with other encapsulated organisms, the primary pathogenetic role of the capsule is to allow the organism to avoid phagocytosis by leukocytes. By itself the capsule does not elicit inflammation.[275] There is some clinical and laboratory evidence that when the concentration of free polysaccharide antigen in the blood is very high, there is spontaneous activation of complement that could result in disseminated intravascular coagulation and shock.[235,124]

7.1.1.2. Cell Wall. The cell wall component of the bacterium appears to play an important role in pathogenesis.[275] Components of the cell wall stimulate the recruitment of leukocytes, enhance endothelial permeability, which leads to local edema formation, and induce the production of cytokines. Thus, cell wall components may be responsible for the pathological picture characteristic of pneumococcal pneumonia and meningitis. Consistent with this model is the belief that treatment with antibiotics that cause cell lysis is associated with the release of cell wall components that may temporarily augment the inflammatory response.[275]

7.1.1.3. Surface Proteins. These include PspA and PspC. The former is a surface protein found on all pneumococci that has been shown to be required for full virulence of pneumococci.[34,35] The function of PspAappears to be to reduce consumption of complement by pneumococci which may lead to reduced complement-mediated phagocytosis and clearance of pneumococci. The importance of these surface proteins is that they are highly immunogenic and it has been suggested that they may represent possible ideal protein candidates for alternative pneumococcal conjugate vaccines.

7.1.1.4. Cell Wall Hydrolases/Autolysins. The cell wall autolysins, in particular lytA, are important for the virulence of pneumococci. The mechanisms by which these enzymes contribute to pathogenicity are first by releasing cell wall breakdown products, which, as described above, are known to elicit a wide range of proinflammatory activities, in the host. Second these autolysins release pneumolysin, which is a cytoplasmic protein, released into the external medium when pneumococci undergo autolysis, and which, as described below, is considered to be one of the most important virulence factors of the organism.[221,184]

7.1.1.5. Toxins. The microorganism also has a number of toxins, including pneumolysin, hyaluronidase, neuraminidase, and IgA 1 protease. One of the best studied toxins of the pneumococcus is pneumolysin, a thiol-activated pore-forming toxin.[62] Pneumolysin is a protein consisting of 471 aminoacids and is produced by all clinically significant isolates of pneumococci. It has a number of significant activities that appear to play an important role in the pathogenesis of pneumococcal infections. The toxin has the ability to slow ciliary beating[103,105] and subsequently cause damage to the structural integrity of the ciliated epithelium. The slowing of ciliary beating appears to aid initial colonization of airway epithelium with subsequent invasion occurring following damage to the airway epithelium. Recombinant pneumolysin has also been shown in an experimental model to induce the salient histological features of pneumococcal pneumonia in the rat lung in vivo.[104] The toxin also interacts with a number of components of the hosts' immune response, as described below,[62] which may also play a role in the pathogenesis of pneumococcal infec-

tions. For example, the toxin has been reported to induce apoptosis in a number of cell types, including neutrophils, macrophages, and neuronal cells, which may contribute to persistence of the pathogen. In sub-lytic concentrations the toxin also has been reported to possess a wide range of proinflammatory activities, involving primarily neutrophils and macrophages/monocytes and their inflammatory mediators. Importantly it has recently been reported that exposure of neutrophils to pneumolysin was accompanied by influx of Ca^{2+}, activation of NFkappaB, and synthesis of IL-8.[63,107] Based on several experimental studies, these proinflammatory activities appear to be operative in vivo.

Among the other toxins produced by the organism, hydrogen peroxide (H_2O_2) has also been shown to cause slowing of ciliary beating and damage to the structural integrity of the ciliated epithelium, which neither enhances nor antagonizes these same effects induced by pneumolysin.[99]

Virtually all strains of pneumococci produce hyaluronidase which is said, because of its ability to degrade connective tissue, facilitates tissue invasion by the pneumococcus. This toxin is therefore sometimes called the "spreading factor". However, hyaluronidase also enhances the ciliary slowing and epithelial dysfunction induced by pneumolysin.[100] In addition, it has been said to be able to breach the blood–brain barrier, and both human and experimental studies suggest it plays an important role in the pathogenesis of pneumococcal meningitis.

One or more neuraminidases appear to be produced by clinical isolates of pneumococci, which have been thought to play an important role in the pathogenesis of pneumococcal meningitis. These enzymes have the ability to cleave sialic acid residues from a wide variety of glycolipids, glycoproteins, and oligosaccharides on cell surfaces which might unmask receptors for putative pneumococcal adhesins.[221]

7.1.2. Host Defenses. In humans, host defenses against the pneumococcus can be divided into natural and acquired. Some of the natural host defenses have been deduced from experimental studies, but to a large extent they have been inferred from high rates of disease in people with underlying (predisposing) abnormalities.

The natural defenses in individuals with normal lungs include the transport of bacteria out of the lungs by the coordinated motion of the cilia and the production of an inflammatory response.[95,96] Damage to the lungs by previous infections, smoking, bacterial toxins (as described above), occupational exposure to lung toxins, or chronic obstructive pulmonary disease is associated with defective clearance by these mechanisms and therefore is associated with enhanced susceptibility to infection by *S. pneumoniae*.

Humoral immunity is of considerable importance in acquired resistance to infection with the pneumococcus. Individuals whose B-cell function is abnormal are predisposed to infection on this basis. This includes infants, children, and adults who have congenital B-cell deficiency and hypogammaglobulinemia. This predisposition can be ameliorated by treatment with intramuscular or intravenous gamma globulin every 3–4 weeks. Acquired dysgammaglobulinemias including multiple myeloma and acquired immunodeficiency syndrome (AIDS) are associated with unusual susceptibility to infection by the pneumococcus. Children and adults with HIV infection are highly susceptible to pneumococcal infections.[26,160,6,89,150] Children with AIDS treated prophylactically with intravenous immune globulin have significantly fewer bacterial infections, especially pneumococcal infections.[270]

The presence of a normal spleen is important in containing pneumococcemia. In persons with anatomical or functional asplenia, there is a high risk of overwhelming sepsis. Congenital asplenia is often part of a complex of midline defects and is termed Ivemark's syndrome.[156] Individuals with this syndrome frequently die from severe cardiac disease in infancy. Of those who survive past 6 months of age, sepsis is a frequent occurrence and *S. pneumoniae* is most frequently the cause of sepsis.[278] Of those who have a splenectomy in later life, overwhelming pneumococcal sepsis is a significant threat. Posttraumatic splenectomy may be associated with a lower risk than elective surgical splenectomy because splenic rupture is often associated with splenosis (implantation of functioning spleen cells in the abdominal cavity). In the absence of splenosis, susceptibility to overwhelming pneumococcal sepsis is lifelong. The characteristic syndrome of overwhelming postsplenectomy infection is associated with *S. pneumoniae* in 50–90% of cases with a mortality rate of 50–70% despite treatment with appropriate antibiotics.[260]

The condition most clearly associated with acquired hypofunction of the spleen and hypersusceptibility to pneumococcal infection is sickle-cell anemia. In this disease the spleen is normal in the early months of life and then develops progressive autoinfarction due to stasis of sickled red cells. Finally, there is loss of splenic function even though the spleen may be anatomically present. This is the time of maximum susceptibility to life-threatening pneumococcal infection. The threat of pneumococcal sepsis and meningitis may be decreased by the use of prophylactic penicillin, or in those over 2 years of age, pneumococcal vaccine.[223,119]

The complement system has an important role in the pathogenesis of pneumococcal infections. Using animal models, it has been possible to demonstrate that opsonization of pneumococci, which is necessary for eradication of bacteremia, requires participation of the complement system.[41] Pneumococci can activate the terminal complement

components via the classical or alternative complement pathways. The alternative complement pathway can be activated by pneumococci and immunoglobulin that does not have to be specific for pneumococcal antigens. This pathway is responsible for "natural immunity" in non-immune individuals and activates opsonic C3b. Activation of the classical complement pathway is more efficient and occurs in immune individuals who have specific antipneumococcal antibody in the serum. It also involves activation of C3b. Hypersusceptibility to pneumococcal infection may be a consequence of dysfunction of either system. With congenital absence of any of the early components of the complement cascade, there is frequently recurrent infection, especially with pneumococci. In sickle-cell disease there is abnormally low function of the alternative complement system and this appears to be part of the susceptibility to overwhelming pneumococcal infections.[291]

7.2. Immunity

Type-specific anticapsular antibody appears on the fifth or sixth day of illness in untreated patients and promotes the ingestion of bacteria by leukocytes. The antibody appears to be protective and long-lasting and is specific for the pneumococcal capsular type. Recurrent systemic infection due to the same serotype is rare except in patients with impaired defenses or persistent foci of infection. However, the pneumococcus may persist in the upper-respiratory tract for days or weeks in the presence of humoral antibody.

Type-specific capsular antibodies also appear about 11 days after immunization, peak in 3–4 weeks, and persist at protective levels for at least 5 years.[238] Numerous studies have demonstrated that antibody production in response to pneumococcal polysaccharide vaccine is poor below the age of 2 years and is probably not effective in preventing infection in children of that age.[78,241] While type-specific immunity explains the success of the polysaccharide and conjugate vaccines, it does not explain the major decline in invasive pneumococcal disease that occurs around age 5[178] as young children could not have been exposed to the range of pneumococcal types by that age. Recent animal data suggest that antibody-independent mechanisms may explain immunity to pneumococcal carriage.[192]

8. Patterns of Host Response

8.1. Clinical Features

Pneumococcal infections in humans are manifested in various ways (Table 4). These range from the healthy carrier state or minor respiratory infection to pneumonia and meningitis, otitis media, conjunctivitis, and other focal purulent infections. Bacteremia may occur in association with manifestations ranging from simple pharyngitis and fever without a demonstrably localized lesion[198,265] to fulminating septicemia with purpura and adrenal hemorrhage (Waterhouse–Friderichsen syndrome), which may be rapidly lethal in the immunocompromised or asplenic patient.[279] Pneumococcal meningitis is most commonly a complication of skull fracture, sinusitis, mastoiditis, or pneumonia. Pneumococcal empyema of the pleura is a fairly common complication of pneumococcal pneumonia, which appears to be increasing in incidence, as described above, and infective endocarditis is generally a late complication.

Table 4. Clinical Spectrum of Pneumococcal Infections

Minor respiratory infections	Meningitis
Conjunctivitis	Focal infections
Otitis media	Febrile bacteremia
Sinusitis	Septicemia
Lobar pneumonia	Empyema of pleura
Bronchopneumonia	Endocarditis

Clinically, pneumococcal lobar pneumonia begins characteristically, most often after a few days of mild upper-respiratory symptoms, with a sudden severe chill or pleuritic pain or both. This is accompanied by a hacking cough, with production of small amounts of orange-, rust-, or prune-juice-colored sputum, and followed by fever and the appearance of physical and X-ray signs of consolidation of the lung(s). If the condition is untreated, the organism may invade the bloodstream (bacteremia), and the consolidation spreads to involve an entire lobe. It may spread to other parts of the lung, with a stormy course that ends in death or in recovery by rapid drop in fever and cessation of symptoms (crisis) or by slow resolution of fever, symptoms, and signs over several days (lysis).

In some areas of the world, such as sub-Saharan Africa, many of the patients presenting with pneumococcal infections are found to have concomitant HIV infection. In many of these patients the pneumococcal pneumonia is the presenting feature of the HIV infection, such that it has been recommended that patients presenting with invasive pneumococcal pneumonia should be offered counseling and testing for HIV infection. Investigators have studied the clinical presentation of bacteremic pneumococcal pneumonia, comparing HIV-seropositive and HIV-seronegative cases, and have noted some differences in presentation.[101] HIV-seropositive patients are younger, with a higher ratio of female patients. While some differences werenoted in the routine laboratory

parameters (lower admission white cell counts and serum albumin levels in the HIV-seropositive patients) and the microbiological data (more penicillin-resistant isolates and more of the so-called childhood serotypes/groups (6, 14, 19, 23) in the HIV-seropositive patients), there were no differences noted in the hospital course, complications, or outcome of the two patient groups.[101] However, recent analyses in which adjustments were made for age and severity of illness suggest that HIV-infected patients do, in fact, have a higher mortality, which is related, at least in part, to their level of immunocompromise, as indicated by their CD 4 cell count.[102]

9. Control and Prevention

9.1. Antibiotic Treatment

For many years the treatment of choice for pneumococcal infections remained penicillin to which the organism remained exquisitely sensitive. However, emerging penicillin and other antibiotic resistance has changed the prescribing recommendations and practices of many clinicians throughout the world. Much has been written about the impact of resistance to the various classes of antibiotics and the outcome of infections treated with these antibiotic classes alone. This topic has been reviewed elsewhere.[172,171,97] While it is interesting to note that there have been microbiologically documented failures of many of the different classes of antibiotics, including the cephalosporins, the macrolides, and even the fluoroquinolones, in cases infected with penicillin-nonsusceptible pneumococci, there have been no microbiologically documented failures of parenteral penicillin therapy in adequate dose. A prospective study of invasive pneumococcal infections involving 844 isolates from 22 countries on 6 continents documented no adverse effects of discordanttherapy on the outcome of these infections with the use of the high dose parenteral penicillin therapy or cefotaxime or ceftriaxone. The study did document a negative outcome in cases with cephalosporin-resistant isolates treated with cefuroxime at a dose of 750 mg TID.[294] Since the study of Yu and colleagues in 2003 at least four more recent studies appear to have confirmed the apparent lack of impact of antibiotic resistance on the outcome of most cases with antibiotic-resistant pneumococcal pneumonia, leading to the current increase in breakpoints for penicillin resistance in non – meningeal infections.[11,108,254,32]

More recently several studies investigating the impact of antibiotic therapy on outcome of bacteremic pneumococcal infections have suggested that the outcome of such infections is improved by the use of combination antibiotic therapy, most commonly that of a macrolide/azalide combined with standard beta-lactam therapy.[201,281,193,19,283,280] The reason for the benefit of combination therapy on outcome is uncertain and may well be multifactorial in origin. Suggested reasons include the presence of infections with the so-called "atypical pathogens" (*Chlamydia* spp., *Mycoplasma* spp., or *Legionella* spp.) requiring specific treatment with a macrolide/azalide, antimicrobial resistance of the isolates to one of the antimicrobial agents, the possibility of polymicrobial infections, antibiotic synergism, particularly occurring in immunocompromised patients and the non-antimicrobial, immunomodulatory, anti-inflammatory activity of the macrolide/azalide group of antibiotics.[280]

9.2. Prevention

Although controversy about the efficacy of active immunization with a polyvalent vaccine persists, it has been the mainstay for the prevention of invasive pneumococcal infections. However, investigators have also used passive immunization with specific immune intravenous immunoglobulin preparations in an attempt to decrease the mortality of critically ill patients with severe pneumococcal infections, without apparent benefit (Charles Feldman, personal communication).

9.3. Pneumococcal Polysaccharide Vaccine

Because of the extremely high incidence of serious pneumococcal infections among South African goldminers,[194] Wright and his co-workers in 1911[292] immunized miners with a crude whole-cell pneumococcal vaccine. Subsequently, a number of other investigators conducted clinical trials of the safety and efficacy of polysaccharide vaccines against pneumococci of various serotypes.[181,85,106] The validity of the results of many of these trials was questionable because of such methodological flaws as non-randomization, inadequacies in the clinical follow-up of subjects, and inadequacies in the bacteriological confirmation of the outcomes. Controlled trials of bivalent, trivalent, and quadrivalent polysaccharide vaccines that were conducted in the 1940s provided stronger evidence that vaccines were efficacious.[187,165] Two different hexavalent vaccines subsequently were commercially produced and marketed. At about the same time, antimicrobials that were effective against pneumococci became available and the outcomes of patients with pneumococcal infections improved substantially.

Interest in the vaccines rapidly diminished and they were eventually withdrawn from the market.

In 1964, Austrian and Gold[17] reported a mortality rate of 25% in adults with bacteremic pneumococcal pneumonia. Many of these patients died despite early treatment with appropriate antibiotics. Furthermore, most of the patients were either elderly or had a chronic illness which represented a population at increased risk of serious pneumococcal disease. This population could be targeted for immunization if an effective vaccine could be developed. Accordingly, Austrian and others worked together to develop a polyvalent vaccine.[18] The vaccine was tested with prospective, double-blind, randomized, controlled trials in young South African goldminers. This group of subjects was chosen because of the extraordinarily high incidence of pneumococcal disease they experienced, which enabled these trials to be conducted expeditiously and at a relatively low cost.[18]These well-designed trials produced conclusive evidence of the efficacy of these vaccines in this population (the estimates of protective efficacy ranged from 76 to 92%).[18,253]

Two large clinical trials were conducted to try to assess the vaccine's efficacy in populations at high risk of serious pneumococcal disease in the United States.[13] These trials were conducted among 13,600 ambulatory patients over 45 years of age at the Kaiser-Permanente Health Plan in San Francisco and among 1,300 subjects at a chronic-care facility in North Carolina. Despite the relatively large number of subjects, the results were inconclusive because of the low incidence of invasive pneumococcal infections and the consequent limited statistical power of these trials.[13,240] A small clinical trial conducted among patients with sickle-cell disease found excellent efficacy for these patients, but the study was flawed because the trial was neither randomized nor blinded.[4]

Despite these equivocal results, a 14-valent vaccine that contained 50 μg each of the polysaccharide antigen of 14 serotypes of pneumococci was licensed by the FDA in 1977. This vaccine was replaced in 1983 by one containing polysaccharide antigens of 23 capsular types (Table 3). The vaccine was recommended for use in persons over 2 years of age with a variety of chronic conditions that placed them at increased risk of serious pneumococcal infection.[54] These conditions included anatomical or functional asplenia and chronic pulmonary, cardiac, or renal diseases. The recommendations for the exact target population were rather vague, in part because of a lack of firm data on the magnitude of the increased risk of serious pneumococcal infections associated with various chronic conditions. The most recent recommendations from the Immunization Practices Advisory Committee (ACIP) of the CDC are as follows:[56,57]

Adults

1. Immunocompetent adults who are at increased risk of pneumococcal disease or its complications because of chronic illnesses (e.g., cardiovascular disease, pulmonary disease, diabetes mellitus, alcoholism, cirrhosis, or cerebrospinal fluid leaks) or who are ≥ 65 years old.
2. Immunocompromised adults at increased risk of pneumococcal disease or its complications (e.g., persons with splenic dysfunction or anatomic asplenia, Hodgkin's disease, lymphoma, multiple myeloma, chronic renal failure, nephrotic syndrome, or conditions such as organ transplantation associated with immunosuppression).
3. Adults with asymptomatic or symptomatic HIV infection.

Children

1. Children ≥ 2 years old with chronic illnesses sporadically associated with increased risk of pneumococcal disease or its complications [e.g., anatomic or functional asplenia (including sickle cell disease), nephrotic syndrome, cerebrospinal fluid leaks, and conditions associated with immunosuppression].
2. Children ≥ 2 years old with asymptomatic or symptomatic HIV infection.
3. The currently available 23-valent vaccine is *not* indicated for patients having only recurrent upper-respiratory-tract disease, including otitis media and sinusitis.

Special Groups

1. Persons living in special environments or social settings with an identified increased risk of pneumococcal disease or its complications (e.g., certain Native American populations).

Adverse Reactions

1. Approximately 50% of persons given pneumococcal vaccine develop mild side effects, such as erythema and pain at the injection site. Fever, myalgia, and severe local reactions have been reported in <1% of those vaccinated. Severe systemic reactions, such as anaphylaxis, rarely have been reported.

Precautions

1. The safety of pneumococcal vaccine for pregnant women has not been evaluated. Ideally, women at high risk of pneumococcal disease should be vaccinated before pregnancy.

Timing of Vaccination

1. When elective splenectomy is being considered, pneumococcal vaccine should be given at least 2 weeks before the operation, if possible. Similarly, for planning cancer chemotherapy or immunosuppressive therapy, as in patients who undergo organ transplantation, the interval between vaccination and initiation of chemotherapy or immunosuppression should also be at least 2 weeks.

Revaccination

1. In one study, local reactions after revaccination in adults were more severe than after initial vaccination when the interval between vaccinations was 13 months. Reports of revaccination after longer intervals in children and adults, including a large group of elderly

persons revaccinated at least 4 years after primary vaccination, suggest a similar incidence of such reactions after primary vaccination and revaccination.

2. Without more information, persons who received the 14-valent pneumococcal vaccine should not be routinely vaccinated with the 23-valent vaccine, as increased coverage is modest and duration of protection is not well defined. However, revaccination with the 23-valent vaccine should be strongly considered for persons who received the 14-valent vaccine if they are at highest risk of fatal pneumococcal infection (e.g., asplenic patients).
3. Revaccination should also be considered for adults at highest risk who received the 23-valent vaccine 6 years before and for those shown to have rapid decline in pneumococcal antibody levels (e.g., patients with nephrotic syndrome, asplenia, or sickle-cell anemia who would be ≤10 years old at revaccination).

New recommendations from the Advisory Committee on Immunization Practices for the prevention of pneumococcal disease were issued in April, 1997. The major change is that revaccination with polyvalent pneumococcal polysaccharide vaccine is recommended for patients 65 years of age or older who received the vaccine five or more years earlier and were <65 years of age when they last received the vaccine. Reimmunization also is recommended both for persons with either functional or anatomic asplenia and for immunocompromised persons if 5 or more years have elapsed since the last dose (for persons <11 years of age, if 3 or more years have elapsed since the last dose).

Strategies for Vaccine Delivery

1. Recommendations for pneumococcal vaccination have been made by the ACIP, the American Academy of Pediatrics, the American College of Physicians, and the American Academy of Family Physicians. Recent analysis indicates that pneumococcal vaccination of elderly persons is cost-effective. The vaccine is targeted for approximately 27 million persons aged ≥ 65 years and 21 million persons ages <65 years with high-risk conditions. Despite Medicare reimbursement for costs of the vaccine and its administration, which began in 1981, annual use of pneumococcal vaccine has not increased above levels observed in earlier years. In 1985, <10% of the 48 million persons considered to be at increased risk of serious pneumococcal infection were estimated to have ever received pneumococcal vaccine.
2. Opportunities to vaccinate high-risk persons are missed both at time of hospital discharge and during visits to clinicians' offices. Two thirds or more of patients with serious pneumococcal disease had been hospitalized at least once within 5 years before their pneumococcal illness, yet few had received pneumococcal vaccine. More effective programs for vaccine delivery are needed, including offering pneumococcal vaccine in hospitals (at the time of discharge), clinicians' offices, nursing homes, and other chronic-care facilities. Many patients who receive pneumococcal vaccine should also be immunized with influenza vaccine.

The recommendation for use in children 2–5 years of age at increased risk of invasive pneumococcal infection has been modified[58] to include conjugate vaccine as follows: two doses of conjugate vaccine ≥ 2 months apart followed by 23-valent vaccine ≥ 2 months later. Children who have already received polysaccharide aged 2–5 years should receive two doses of conjugate as above, ≥ 2 months after the polysaccharide. Because of potential concerns about the induction of tolerance, polysaccharide vaccines should follow, not precede conjugate immunizations.

Based on systematic surveillance of the serotypes of pneumococci that caused serious invasive disease, the vaccine was formulated to include serotypes that were responsible for approximately 80% of the cases of systemic pneumococcal infections in the United States.[39,40,38] However, soon after the vaccine was licensed and distributed, reports of clinical failures of the vaccine began to appear.[1,126] Furthermore, studies suggested that some high-risk patients had poor antibody responses to the vaccine.[175,5,125] In addition, surveillance data from the CDC suggested that vaccine efficacy was poor for many high-risk patients.[39,37] Thus, there was considerable uncertainty about the vaccine because it was shown to be effective in a very select population (young goldminers), but there were serious questions about its efficacy for the population in the United States that was at increased risk of serious pneumococcal infections. However, because of the low incidence of bacteremic pneumococcal infections in developed countries, even in high-risk patients, there are formidable logistical and financial obstacles to conducting a randomized clinical trial in the United States.[60] Furthermore, because the vaccine is licensed (and presumably is efficacious), it may be unethical to allocate high-risk patients randomly to receive placebo, as would be necessary in a randomized trial.

Alternatives to randomized clinical trials to assess the vaccine's efficacy were necessary.[60] Shapiro and Clemens[245] conducted a case–control study that found the vaccine's efficacy to be 67% (95% confidence interval: 13–87%) in high-risk patients. Although there were too few subjects to assess the vaccine's efficacy accurately in immunocompromised patients, its efficacy in high-risk patients with chronic conditions such as diabetes mellitus or chronic pulmonary, cardiac, or renal disease was 77% (95% confidence interval: 27–93%). Bolan and colleagues[31] from the CDC extended their estimates of the vaccine's efficacy based on their surveillance data of reports of serotypes of pneumococci that caused bacteremic disease from 1978 to 1984. They found the vaccine's efficacy against bacteremic disease to be 64% (95% confidence interval: 47–76%). Based on these results, experts have concluded that the vaccine is cost-effective and that it is underutilized.[56,222,16,174,249]

Despite the serious obstacles to such a study, Simberkoff and colleagues[246] conducted a randomized clinical trial of the protective efficacy of the 14-valent vaccine at several different Veterans Administration medical centers. Although they were unable to demonstrate that the vaccine was efficacious, there were a number of serious flaws in their methodology that compromise the validity of their conclusions.[243]

Many of the events that the investigators counted as episodes of pneumococcal infection were really of uncertain cause since their definition of pneumococcal infection included patients with a cough and isolation of pneumococci from their sputa (even in the absence of an abnormal chest X-ray). Because it is known that immunization with pneumococcal vaccine will not affect colonization of the respiratory tract with pneumococci, the definitions of pneumococcal infection used in this study limit its usefulness. By the investigators' own criteria, there were only two subjects with proven (bacteremic) pneumococcal infection. As a result, the statistical power of the study to assess the vaccine's efficacy against proved pneumococcal infections was extraordinarily poor (only 6%).

Despite the recommendations of official bodies such as the ACIP[56] and the American College of Physicians[143] that pneumococcal vaccine be administered to high-risk patients, physicians have been slow to implement these recommendations. Studies have indicated that fewer than a quarter of the eligible patients have received the vaccine.[245,90] Consequently, one of the major issues in maximizing the full potential benefit of this vaccine is to find a strategy to increase its utilization among high-risk patients. Fedson[90] and others have found that a large majority of the patients who develop pneumococcal bacteremia had been hospitalized in the preceding several years. Consequently, he suggests a strategy of immunizing with pneumococcal vaccine hospitalized patients who have indications for the vaccine.[90]

The most definitive postlicensure study of the vaccine's protective efficacy is a case–control study by Shapiro et al.,[244] conducted from 1984 to 1990, of 1,054 adults with systemic (usually bacteremic) pneumococcal infection who had one or more indications for pneumococcal vaccine and 1,054 matched controls. The case subjects were stratified into a very high risk, immunocompromised group (18% of the cases) and an immunocompetent group (82% of the cases). The estimate of the vaccine's aggregate protective efficacy (against serotypes in the vaccine) was 61% (95% confidence interval: 47–72%; $p < 0.00001$) among the immunocompetent patients but was only 21% (95% confidence interval: 55–60%; $p = 0.48$) for the immunocompromised group. Although this was an observational study, the findings were robust and did not change after statistical adjustment for potential confounders. In addition, using the same methods, the investigators found that the vaccine was not efficacious in preventing infections caused by serotypes not included in the vaccine and that there was no statistically significant difference in the proportion of cases and controls who had received influenza vaccine. These observations help assure that the findings were not substantially affected by bias and that the conclusions of the investigators were valid.

A report from the CDC in which the vaccine's protective efficacy was assessed based on an indirect cohort method had results similar to those of Shapiro and co-workers.[244] In that study (based on isolates of pneumococci from normally sterile sites that were submitted to the CDC from 1978 to 1992) the investigators found that the vaccine was efficacious in immunocompetent persons with indications for the vaccine (estimates of efficacy ranged from 65 to 75%, depending on the underlying disease), but that it was not efficacious (although the confidence intervals around the estimates were wide) for severely immunocompromised patients such as those with sickle-cell disease, lymphoma, or multiple myeloma.[50]

An area of particular controversy that has not been resolved is whether there is a significant decline in the efficacy of the vaccine over time. Although there does seem to be some decline, the magnitude of the decline (at least over 5–8 years) is relatively modest and some of this decline may be attributable to increased cumulative risk over time of exposure to serotypes of pneumococci in the vaccine. It is not clear that revaccination will reverse this effect. Nevertheless, the risk of adverse side effects from revaccination is small and is related to how soon after the first vaccination it occurs (if >1 year after the first vaccination, the risk of severe adverse reactions is extremely small). Consequently, revaccination is recommended for those at particularly high risk of serious pneumococcal infections.

The vaccine has been shown in case–control studies to be protective in HIV-infected patients in the US – most of these patients, however, are receiving HAART. The risk of pneumococcal disease in the HAART era remains elevated[133,144] and the recommendation in favor of the vaccine seems reasonable in this population. In populations of HIV-infected people without access to HAART, the vaccine has been shown not to be effective[117] and may in fact be harmful, presumably on the basis of committing memory B cells to the vaccine in individuals unable to mount an immune response or to replenish their memory B cells. The vaccine has not been shown to reduce non-bacteremic pneumonia in randomized trials in the elderly.[157]

The type-specific pneumococcal polysaccharide appears to be a largely T-cell-independent antigen. Booster (anamnestic) responses to repeated exposure to the polysaccharide antigen are not observed.[33] Although the attack rate of invasive pneumococcal infection is very high in young children, most of the serious infections occur in children under 2 years of age. Unfortunately, children under 2 years of age have a poor immunologic response to T-cell-independent antigens such as those in pneumococcal vaccine.

Consequently, universal immunization of children with pneumococcal polysaccharide vaccine was not recommended, since most cases of serious disease occur among young children who are unlikely to respond to the vaccine.[122] The vaccine also has not been effective in preventing otitis media in young children.[122] Pneumococcal vaccine is indicated to booster conjugate vaccine for children 2 years of age or older with chronic conditions that place them at increased risk of serious pneumococcal infections.[46,122]

Although there continues to be some controversy about the protective efficacy of polyvalent pneumococcal polysaccharide vaccine for adults, there is little doubt that it is not efficacious in most children <2 years of age – the group at highest risk of serious pneumococcal infections.

9.4. Pneumococcal Conjugate Vaccines

In the year 2000, the first 7-valent pneumococcal conjugate vaccine was licensed. The 7-valent vaccine (Table 3) contains serotypes 4, 6B, 9 V, 14, 18C, 19F, and 23F (a 9-valent version includes additionally types 1 and 5; a 10-valent version also contains serotype 7, which is a relatively common cause of otitis media; a 13-valent version includes also serotypes 6A, 7F, and 19A, the first and last of which are important antibiotic-resistant types). The serotypes in the 7-valent vaccines are responsible for 80–85% of invasive pneumococcal infections (and for about 75% of the cases of pneumococcal otitis media) in the United States. Addition of other serotypes, such as serotypes 1 and 5, will be important to increase the proportion of serious infections that might be prevented by the vaccine in developing countries.[21] These vaccines have been shown to be immunogenic in infants and primes them for anamnestic antibody responses to a booster dose of either the conjugate or the plain polysaccharide vaccine.[166,155]

Pneumococcal 7-valent vaccine conjugated to diphtheria protein CRM_{197} has been shown in large double-blind randomized trials in California[28] and the Navajo[208] to prevent >85% of invasive disease due to vaccine serotypes. Similar levels of efficacy have been found using the same vaccine in a 9-valent formulation including serotypes 1 and 5 in Africa.[173,71] Vaccine efficacy has also been established in one of these studies among HIV-infected infants with protection against invasive disease of 65%.[173] In contrast to the lack of evidence for protection based on randomized trials using 23-valent vaccine in the elderly, clinical trials of the conjugate vaccines do show prevention of pneumonia in young children.[29,173,71] Conjugate vaccines can thus be used to define the vaccine-preventable fraction of pneumonia.[190] These studies have shown that a significant fraction of vaccine-preventable pneumonia in children is not associated with consolidation on X-ray and that adjunctive tests such as a raised CRP (>40 mg/dl) may be a useful predictor of a pneumococcal etiology.[189] Further, the conjugate vaccine as a probe has found that 45% of influenza-associated pneumonias in African children may be pneumococcal.[188] These data suggest a major role for conjugate vaccine in preventing influenza-associated morbidity due to pneumococcal superinfection. Much of the pneumonia associated with other respiratory viruses such as RSV, parainfluenza viruses, and human metapneumovirus may also be prevented in children who have received pneumococcal conjugate vaccine.[188]

The recommendations for pneumococcal conjugate vaccines according to the ACIP[58] are

Children for Whom PCV7 Is Recommended

Children Aged≤ 23 Months

All children aged ≤ 23 months should be vaccinated with PCV7 (Strength of evidence: children aged 2–6 months, A; children aged 7–23 months, B)

Vaccination Schedule. Infants receiving their first dose at age ≤ 6 months should receive three doses of PCV7 at intervals of approximately 2 months, followed by a fourth dose at age 12–15 months. Newborns should begin the schedule at age 2 months, although PCV7 can be administered as young as age 6 weeks. Prematurely born infants (i.e., <37 weeks gestation) should receive PCV7 at the recommended chronologic age concurrent with other routine vaccinations. For infants with prolonged nursery stays, initiation of vaccination should begin during discharge planning. Children aged ≥7 months not previously vaccinated should also be vaccinated according to the recommended schedule. The proposed vaccination schedule is the same for all children aged ≤23 months, regardless of the presence of underlying medical conditions (e.g., children with HIV infection, SCD or other asplenia, chronic disease, or who are otherwise immunocompromised). Interruption of the vaccination schedule does not require reinstitution of the entire series or the addition of extra doses.

Children Aged 24–59 Months Who Are at High Risk for Pneumococcal Infection

Children aged 24–59 months should receive PCV7 vaccination if they are at high risk for pneumococcal infection caused by an underlying medical condition. This recommendation applies to the following groups:

- children with SCD and other sickle cell hemoglobinopathies, including hemoglobin SS, hemoglobin S-C, or hemoglobin S-β-thalassemia, or children who are functionally or anatomically asplenic;
- children with HIV infection;
- children who have chronic disease, including chronic cardiac and pulmonary disease (excluding asthma), diabetes mellitus, or CSF leak; and
- children with immunocompromising conditions, including a) malignancies (e.g., leukemia, lymphoma, Hodgkin's disease); b) chronic renal failure or nephrotic syndrome; c) those children receiving immunosuppressive chemotherapy, including long-

term systemic corticosteroids; and d) those children who have received a solid organ transplant.

SCD and HIV (Strength of evidence: B). Chronic disease or other (Strength of evidence: C).

Vaccination Schedule. For children aged 24–59 months with underlying medical conditions ACIP recommends two doses of PCV7, administered 2 months apart, followed by one dose of PPV23 administered ≥2 months after the second dose of PCV7. Penicillin prophylaxis should be continued for children with SCD to age ≥5 years, regardless of vaccination with PCV7.

Other Children Who Might Benefit from Vaccination with PCV7

ACIP recommends that health-care providers consider PCV7 vaccination for all other children aged 24–59 months, with priority given to the following populations:

- children aged 24–35 months;
- children of Alaska Native or American Indian descent;
- children of African-American descent;
- children who attend group day care centers;

(Strength of Evidence: B).

If pneumococcal vaccine is to be used among healthy children aged 24–59 months, ACIP recommends that PCV7 be used.

Vaccination Schedules. ACIP recommends that one dose of PCV7 be considered for Alaska Native and American Indian children aged 24–59 months. Vaccination program personnel and other health-care providers might consider whether Alaska Native and American Indian children aged 24–59 months would benefit by the additional coverage provided by the 23-valent polysaccharide vaccine. ACIP recommends that physicians consider administering one dose of PCV7 to their African-American pediatric patients aged 24–59 months because of their increased risk for pneumococcal infection. No additional vaccination with PPV23 is recommended (Strength of evidence: B). Additionally, because of increased risk for invasive pneumococcal disease, colonization with antibiotic-resistant pneumococcal strains, and AOM, health-care providers should consider administering one dose of PCV7 to previously unvaccinated children aged 24–59 months who attend group day care centers.

Children Aged ≥5 Years and Adults Who Are At High Risk for Pneumococcal Infection

Administering PCV7 to older children with high-risk conditions is not contraindicated. Current data do not support a recommendation to replace PPV23 with PCV7 among older children and adults.

Recommendations for Use of PCV7 Among Children Previously Vaccinated with PPV23

Children aged 24–59 months who are at high risk for pneumococcal disease and who have already received PPV23 (i.e., children with SCD, HIV infection, or who have other immunocompromising illnesses or chronic diseases) could benefit from the immunologic priming and T-cell-dependent immune system response induced by PCV7. Thus, among children in these groups at high risk, sequential use of the two pneumococcal vaccines can provide additional protection. Health-care providers should vaccinate children aged 24–59 months at high risk who have not previously received PCV7 but who have already received PPV23 with two doses of PCV7 administered ≥2 months apart. Vaccination with PCV7 should be initiated ≥2 months after vaccination with PPV23. Providers should be aware that minimal safety data are available regarding this vaccine sequence.

Recommendations for Use of PPV23 Among Children Previously Vaccinated with PCV7

Administration of PCV7 Followed by PPV23 Among Children at High Risk for Pneumococcal Disease

Children who have completed the PCV7 vaccination series before age 2 years and who are among risk groups for which PPV23 is already recommended should receive one dose of PPV23 at age 2 years (≥2 months after the last dose of PCV7). These groups at high risk include children with SCD, children with functional or anatomic asplenia, children who are HIV infected, and children who have immunocompromising or chronic diseases. Although data regarding safety of PPV23 administered after PCV7 are limited, the opportunity to provide additional serotype coverage among these children at very high risk justifies use of the vaccines sequentially. For children of Alaska Native or American Indian descent, addition of PPV23 after PCV7 can be considered.

Pneumococcal conjugate vaccines not only prevent invasive disease and pneumonia, but have also been shown to prevent 50–60% of vaccine-serotype otitis media.[86,167] Serotype replacement, however, is a significant problem for otitis media prevention, such that the overall protection against clinical otitis media is limited.[28,86] It is possible that a formulation of the conjugate with protein D of *H. influenzae* may result in less replacement, and due to its impact also on nontypeable haemophilus, may prevent a greater fraction of otitis media.[227] Direct comparison of the efficacy of the two vaccines (CRM_{197} versus *Haemophilus* protein D conjugates) is complicated by a more stringent definition of otitis media in the protein D trial.

These conjugate vaccines also reduce vaccine type carriage,[170,227] although there is clear evidence for serotype replacement among recipients of the CRM_{197}-based conjugates.[170] The impact on colonization appears to be prevention of acquisition of new strains rather than eradication of existing carriage[195] and may be mediated by very high values of circulating IgG that may diffuse onto mucosal surfaces.[73] The major impact of conjugate vaccine on serotype-specific acquisition of carriage is that transmission of pneumococci is interrupted in families[130] and communities.[147] In the United States there has been a dramatic reduction of >65% in invasive disease due to the conjugate vaccine serotypes amongst the elderly, since the introduction of conjugate vaccine in children.[288] Decreases of a similar magnitude have been noted among HIV-infected adults although there is evidence of more replacement disease among HIV-infected people, particularly women.[116]

Finally the conjugate vaccine has been shown to protect children from antibiotic-resistant invasive disease[173]

and from carriage of resistant strains.[195,74] This impact on resistance has also been translated into herd immunity and there have been reductions in antibiotic-resistant bacteremias in the United States following conjugate vaccine introduction.[27,263,256] The reduction in resistance has been less in upper-respiratory tract specimens as a result of replacement and continuing selection of resistance among non-vaccine types.[215]

10. Unresolved Problems

The detailed studies of the pneumococcus have been the basis of modern molecular biology and immunochemistry. Probably more is known about the biology of the pneumococcus than about that of any other bacterium. However, the exact mechanism(s) whereby this organism is able to multiply in animal and human tissue and produce its more or less unique type of inflammation in the lung is not fully known. Some progress has been made in the elucidation of the function of various chemical and morphological constituents of the pneumococcus other than the capsular polysaccharide.[113,270] Although many of the defects in cellular and humoral immunity that predispose to pneumococcal infections have been revealed, there are still others that need to be elucidated. Also, the exact mechanism whereby viral infection and chemical irritants predispose local pulmonary tissues to pneumococcal disease needs further elucidation. The reasons for the predominance of infection in males need to be clarified, as does the increased susceptibility of black people, apart from environmental factors. The basis for virulence of pneumococci needs further study. Finally, while pneumococcal vaccines are currently in use, the extent of their global use in target populations is too low, and the challenge remains to get conjugate or pure protein-based vaccines to the millions of at-risk children and adults currently without access to existing vaccines.

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CHAPTER 30

Q fever

Thomas J. Marrie

1. Introduction

Q fever is the illness in man due to infection with the microorganism *Coxiella burnetii*. It is a zoonosis and its epidemiology is closely linked to the animal reservoirs of the causative microorganism, *C. burnetii*. Important elements of the epidemiology of this infection can be learned from the history of its discovery and early investigations; a consideration of the macro-epidemiology at the level of a country; knowledge about the microorganism and the routes of transmission; the epidemiology of Coxiellosis in animals; and finally exploration of the epidemiology at a local level and the lessons learned from outbreaks.

2. Historical Aspects

Dr. E. H. Derrick, a pathologist and Director of the Laboratory of Microbiology and Pathology at the Queensland Health Department, Brisbane, Australia, was contacted by Sir Raphael Cilento, Director General of Health and Medical Services for Queensland in August 1935.[1] Cilento asked Derrick to investigate an outbreak of an undiagnosed febrile illness among workers at the Cannon Hill abattoir in Brisbane.[1]

Derrick first carefully described the clinical illness. He noted that it lasted 7–24 days and was characterized by fever, headache, malaise, anorexia and myalgia. Blood cultures were negative and serum samples from the affected individuals had no antibodies to influenza, typhus, leptospirosis, typhoid and paratyphoid. He named the illness Q (for query) fever.[2]

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Derrick proceeded to investigate the cause of the outbreak by inoculating blood and/or urine from his febrile patients into guinea pigs. The guinea pigs became febrile and when emulsified tissue from these animals was injected into uninfected guinea pigs they too developed fever. However, despite considerable effort Derrick was unable to isolate a microorganism from the guinea pigs. In October 1936 he decided to enlist the aid of Frank MacFarlane Burnet, a virologist (and later a Nobel laureate), who was working at the Walter and Eliza Hall Institute in Melbourne. He sent a saline emulsion of infected guinea pig liver to Burnet. Burnet and his research associate Mavis Freeman found oval areas about the size of a nucleus in sections of spleen from a mouse that had been inoculated with Derrick's specimen. When these smears were stained by Castaneda's method "bodies which appeared to be of rickettsial nature were found sometimes in enormous numbers".[3]

In the spring of 1935 investigators at the Rocky Mountain Laboratory in Hamilton, Montana were trying to find the etiological agent of Rocky Mountain Spotted Fever. As part of this *Dermacentor andersonii* ticks, collected from the Nine Mile Creek area, were brought to Gordon Davis, one of the investigators at the Laboratory.[2] Davis was able to isolate an organism from these ticks.

McFarlane-Burnet sent his "organism" to NIH and by 1938 it was apparent that the tick agent and the Q fever agent were identical. Herald Rea Cox, a Rocky Mountain Laboratory Scientist, and Davis after a great deal of work were able to grow the Nine Mile Agent in embryonated eggs.

Rolla Dyer (Director of the National Institutes of Health from 1942 to 1950) heard about this discovery and visited the Rocky Mountain Lab to review the data. He was convinced that these workers had isolated a microorganism. A few days later on the train on the way back to Washington he developed fever and a severe retro-orbital headache. The

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Nine Mile/Q fever agent was isolated from his blood.[1] This represents the first example of laboratory-acquired Q fever.

Over the years there have been a series of names for this agent – initially *Rickettsia diaporica* (diaporica, Greek for having the property or ability to pass through – describing the filterability of this agent); then *R. burnetii* was proposed and later it was elevated to a subgenus, *Coxiella*.[1] The agent is now known as *C. burnetii* in honor of Cox and Burnet who both died in 1986.

3. Methodology

3.1. Sources of Data

The data sources used for this chapter included an extensive personal collection of papers and books on Q fever and a Medline search to obtain the latest references on Q fever. In addition unpublished observations made by the author were also included. Surveillance data from the US Centres of Disease Control and Prevention were also used.

3.2. Surveys

Most of the surveys that relate to Q fever are seroepidemiological ones. Data from selected surveys are incorporated throughout the manuscript.

3.3. Laboratory Diagnosis

Coxiella burnetii is highly infectious and only category IV laboratories are licensed to isolate it. However, it can be readily isolated from clinical specimens using tissue culture.

Most clinical laboratories use serology as their diagnostic method of choice for this infection. The reference method is the indirect immunofluorescence test. In this method cells from phase I and phase II microorganisms are used. A titre of $\geq$1:8 is considered positive, although in some countries a higher cutoff is used. (The cut off used depends on the antigen and the population being studied). A fourfold rise between acute and convalescent serum specimens is diagnostic of acute Q fever. In acute Q fever the phase II titer is always higher than the phase I titer. In contrast in chronic Q fever, the phase I titer is equal to or higher than the phase II titer. Very high titers such as $\geq$1:16,384 are seen in chronic Q fever. Other serological methods include the complement fixation and enzyme-linked immunosorbent assay. Molecular diagnosis is being used more frequently. Polymerase chain reaction using a variety of primers can be used to amply *C. burnetii* DNA from clinical specimens. However, such a test is not readily available in most diagnostic laboratories.

4. Biological Characteristics of the Organism

In order to understand the epidemiology of Q fever it is necessary to understand some of the unique aspects of the causative organism. *C. burnetii*, an obligate intracellular microorganism, is a pleomorphic coccobacillus with a gram-negative cell wall[4]; it measures 0.2 $\times$ 0.7 μm and has a developmental cycle in which there is a large and small cell variant.[5] The acid pH of the phagolysosome is necessary for development of *C. burnetii,* since the low pH activates its metabolic enzymes.[6] Following maturation of the large cell variant sporogenesis begins.[7] Spore-like formation explains why *C. burnetii* is so successful as a pathogen. It can survive for 7–10 months on walls at 15–20°C, for more than 1 month on meat in cold storage and for more than 40 months in skim milk at room temperature.[7,8]

Coxiella burnetii undergoes phase variation, which is akin to the smooth to rough transition of the lipopolysaccharide of gram-negative bacteria.[9] In nature and in laboratory animals it exists in phase I state in which organisms react with late (45 days) convalescent guinea pigs sera and only slightly with early (21 days) sera.[9] Studies by Hackstadt[10] and Lukacova et al.[11] have shown that in experimentally infected animals the first antibody production is to *C. burnetii* protein – phase II antigen – and later antibody is produced to *C. burnetii* LPS – phase I antigen.

Understanding phase variation is of more than academic interest in that in man high antibody titers ($\geq$1:800), using an indirect immunofluoresence technique, to phase I antigen are suggestive of chronic Q fever, while phase II antibodies predominate in acute Q fever.[12] There is no morphological difference between phase I and phase II cells although they do differ in the sugar composition of their lipopolysaccarides,[13] their buoyant density in cesium chloride and their affinity for basic dyes. The LPS of *C. burnetii* is non-toxic to chick embryos at doses of $>$80 μg/embryo in contrast to *Salmonella typhimurium* LPS which is toxic in nanogram amounts.[11]

Coxiella burnetii is able to survive in the phagolysosome because it produces the enzyme superoxide dismutase which protects it from host-generated superoxide anion and hydrogen peroxide.[14] In common with *Legionella* spp and *Leishmania* spp *C. burnetii* can inhibit the respiratory burst of phagocytosing neutrophils[15] by producing acid phosphatase.[16]

On the basis of 16s rRNA sequence similarities, *C. burnetii* has been placed in the gamma subdivision of proteobacteria with a distant relationship to *Legionella* and *Wolbachia persica*.[17,18]

The 1,995,275 base pair genome of *C. burnetii* phase I, Nine Mile strain, was recently sequenced.[19] It possesses 29 insertion sequence elements and 83 pseudogenes. Because

C. burnetii can persist in the external environment the absence of pathways for synthesis of storage compounds such as glycogen, trehalose or polyhydroxybutyrate was unexpected.[19]

5. Descriptive Epidemiology

5.1. Epidemiology of Q Fever in Selected Countries

5.1.1. United States. Q fever was not a notifiable disease in the United States until 1999, when, because of concerns about its potential as a biological warfare agent, it was added to the national list of notifiable diseases.[20] In two papers McQusiton et al.[20,21] reviewed the epidemiology of Q fever in the United States from 1948 to present. Between 1948 and 1977 a total of 1,168 cases were reported to the Centers for Disease Control and Prevention, a mean of 58.4 cases per year. They also noted that from 1978 to 1986 there was a decline in the number of reported cases to 28.5 per year. Most of the cases (67%) in this early period originated from California.[21] Careful studies of the epidemiology of Q fever in California led to the conclusions that Q fever was associated with exposure to sheep and goats.[22–26] During the course of these studies *C. burnetii* was recovered from the air of premises having infected goats,[23] from raw milk[24] and from the placentas of infected cows.[25]

During the period 1946–1950 outbreaks of Q fever were observed at meat packing plants in Texas and Chicago.[27–32] During the investigation of these outbreaks Shepard implicated aerosols as the route of transmission of Q fever.[32]

In her most recent paper McQusiton[20] reported that from 1978 to 1999 there were 436 cases of Q fever in the United States, an average of 26 cases per year with a range of 6–41 cases. From 2000 to 2004 there were 255 cases from 37 states, an average of 51 per year, range 18–73 cases. These investigators noted a seasonal effect, with 39% of the cases reported from April through June. Seventy-seven percent were male; the mean age was 50.5 years; median 51 years; 92% were white; 6% black; 2% Asian. The racial distribution of the Q fever cases was proportionally similar to that of the US population. The age distribution of the Q fever cases differed from that of the US population with the Q fever cases increasing with increasing age and peaking in the 50–59 year age group. The overall rate for the United States was 0.28 cases per million population per year. The highest rate was in Wyoming. No cases were reported from Alabama, Alaska, Arkansas, Connecticut and Hawaii. Q fever is not reportable in the following states: Delaware, Iowa, Vermont and West Virginia. Hence no data were given for these states.

In a recent study Kim et al.[33] tested 316 bulk milk tanks throughout the United States using PCR to test for the presence of *C. burnetii* DNA. The milk was collected from January 2001 through December 2002 and 94.3% of the samples were positive.

Karakousis et al.[34] reported a case of chronic Q fever and reviewed all the published cases of chronic Q fever from the United States. They could only find seven such cases, likely a gross underestimate, from 1976 to 2004.

McQusiton[21] noted that sheep and goats in the United States have a higher seroprevalence for *C. burnetii* than cattle. While only one study has been published for each of these animals the seropositivity rate among tested dogs was 53%, cats 8% and horses 25.6%.[21] McQuiston also noted an extensive wildlife reservoir for this organism in the United States.[21] Various studies have shown varying degrees of seropositivity for coyotes, grey foxes, skunks, raccoons, rabbits, deer, mice, bears, birds and opossums.

5.1.2. Germany. Hellenbrand, Breuer and Peterson reviewed the epidemiology of Q fever in Germany from 1947 to 1999.[35] In West Germany, Q fever has been a notifiable disease since 1962 and in East Germany since 1979.[35] Q fever peaked in West Germany in 1964 at 450 cases. The average incidence from 1979 to 1999 was 1.1 cases per million persons per year. The rate was highest in southern German states with the highest rate in Baden–Wurttemberg at 4.1 per million per year. They noted that 40 outbreaks of Q fever in humans had been reported. Sheep were implicated in 24, cattle in 6 and there were two laboratory outbreaks. The seasonality changed over the past decade with a marked decrease in winter outbreaks and an increase in summer outbreaks. They also noted that in contrast to earlier community outbreaks which were almost exclusively rural; recent outbreaks have frequently involved people living in urban areas.[35] The location of recent outbreaks suggests that urbanization of rural areas may be contributing to the increase in Q fever.

In Germany, State Veterinarians are required to report biannually the number of herds in which one or more animals have laboratory-confirmed Q fever. The average number of herds positive was 71 between 1971 and 1979, 328 from 1980 to 1989 and 303 from 1990 to 1998. In 1998, 7.8% of 21,191 cattle tested were positive, while 1.3% of 1,346 sheep and 2.5% of 278 goats were positive.[35]

5.1.3. Australia – Including Post Q Fever Fatigue Syndrome (QFFS). In this, the country where it was first recognized, Q fever continues to thrive. In Australia, 6,479cases of Q fever were diagnosed from 1960 to 1986.[36] From 1991 to July 2006 there were 8368 cases, with a range of 354–869 cases per year.[37] The rate of Q fever in Australia is about 30 per million per year.[38] Males are predominantly

involved, with a male:female ratio of 5.3:1.[39] One of the unique risk factors for Q fever in Australia is kangaroo hunting.[40] Another aspect of Q fever epidemiology that is unique to Australia is the widespread use of a *C. burnetii* vaccine in animals.[41] A large outbreak of Q fever in an abattoir near Adelaide that previously processed bacon pigs but added feral goats to their program prompted the vaccine program. The Henzerling strain of *C. burnetii* prepared according to the Ormsbee protocol (Q vax) was chosen. Potential vaccines were screened for antibodies to *C. burnetii* and those who were negative were skin tested intra-dermally for cell-mediated immunity. If the skin test was negative the vaccine was administered. It took some time for the vaccine to gain acceptance, but by 1993, according to Marmion, good progress had been made and by 2004 most large abattoirs in states other than Tasmania (which has fewer cases) had vaccine programs. From 1993/1994 the number of claims by abattoir workers for Q fever (it is a compensable illness in Australia) declined from 139 per year to 2 in 2003/2004.[41]

Another aspect of Q fever that was first recognized and defined in Australia is that of post Q fever fatigue syndrome.[42] It was also reported about the same time from the United Kingdom.[43] The designation, Q fever fatigue syndrome (QFFS), is now used to describe the protracted state of fatigue that can develop in up to 20% of patients who develop acute infection with *C. burnetii*.[42,44] Q fever fatigue syndrome consists of a constellation of symptoms including fatigue, headaches, sweats, arthralgia, blurred vision, muscle fasciculations and enlarged and painful lymph nodes.[42] In a case–control study 5 years after a large outbreak of Q fever in individuals in a community in the West Midlands, United Kingdom, Ayres et al.[45] found that participants who were diagnosed with acute Q fever during the initial outbreak had more complaints of fatigue, sweats, blurring of vision and dyspnea than their age, sex and geographically matched controls and that 42.3% of the infected individuals fulfilled the CDC criteria for chronic fatigue state. Although not conclusive, there is evidence to suggest that "cytokine dysregulation and immunomodulation from persistence of *C. burnetii*" in the host may be responsible for QFFS.[44,46]

Hatchette et al.[47] prospectively followed 49 patients with goat-related Q fever in Newfoundland. The Short Form 36 Health Survey was used to assess the quality of life. Thirty-eight percent (19/49) who had acute Q fever during the outbreak had persistent symptoms 3 months later and had significantly lower scores on all components of the SF-36 than those who did not have persistent symptoms. Two years after the initial outbreak 30% (10/33) of the participants who had serologic evidence of *C. burnetii* infection continued to report persistent symptoms compared with 4% (1/24) of those who did not have Q fever.[47] Helbig et al.[48] compared variability in phenotype distribution among a range of cytokine and accessory immune response genes in 23 patients with post Q fever fatigue syndrome and in 42 Red Cross blood donors from Adelaide, Australia. They found that there were significantly more variants in the natural resistance-associated macrophage protein gene among the patients compared with the controls. In addition there were significant differences in the IL-10 promoter and in intron 1 of the interferon γ gene. HLA DR-11 was present in 19.6% of the cases and 4.3% of the controls ($p = 0.0014$). All of this indicates that there may be a genetic basis for the post Q fever fatigue syndrome.

Hickie and colleagues[49] enrolled 253 patients with Epstein–Barr virus infection ($n = 68$), Q fever ($n = 43$), Ross River virus infection ($n = 60$) and an unconfirmed group ($n = 82$) in a prospective longitudinal study to determine the frequency of chronic fatigue following each of these acute infections and the factors that predict the fatigue state. The case rates for post-fatigue syndrome at 6 weeks, 3, 6 and 12 months were 35, 27, 12 and 9%, respectively, with no difference in case rates between the initial infective agents. Overall there were 29 cases with post-infective fatigue, one was excluded. The initial infection was EBV 5 (7.3%), Q fever 3 (6.9%), RRV 13 (21.6%) and unconfirmed infection 8 (9.7%). These investigators concluded that aspects of the host response rather than the infecting agent is the likely determinant of the post-infective fatigue syndrome.

It is noteworthy, however, that the author of this chapter has treated approximately 300 patients with Q fever pneumonia in Nova Scotia secondary to exposure to infected parturient cats. None of these persons developed QFFS. In the same province one person, following exposure to soil contaminated by goat manure, developed severe QFFS. Thus it is likely that there may be organism, vector and human host factors involved in whether or not QFFS is a sequela of acute Q fever.

The cost of Q fever to the Australian economy has been estimated at $1 million annually and there is a loss of more than 1,700 weeks from work.[39]

5.1.4. France – Including Age and Sex Distribution. The WHO Rickettsial Laboratory under the direction of Didier Raoult in Marseille has been a source that has added greatly to our understanding of not only the epidemiology of Q fever but also the basic science of this infectious disease.

Countrywide studies of the epidemiology of Q fever in France have not been preformed. Nevertheless it is apparent that France (especially the South of France) is one of the countries with the highest incidence of Q fever in the world. In some areas of this country the incidence is 500 per million persons per year.[50] Raoult and coworkers[51] reported their experience with 1,383 cases of

Q fever from 1985 to 1998. They noted that 1,070 (77%) of the cases were acute Q fever, but a remarkable 23% or 313 people had chronic Q fever. Among the acute cases, hepatitis dominated accounting for 40% of the cases; pneumonia and hepatitis 20%; pneumonia 17%; isolated fever 17%; meningoencephalitis 1%; myocarditis 1%; pericarditis 1%; meningitis 0.7%. Most of the patients with chronic Q fever had infective endocarditis 259/313 (83%); 25 had infection of a vascular aneurysm or prosthesis, 15 had Q fever during pregnancy and 7 had osteoarticular infection. They noted that hepatitis occurred in younger patients and pneumonia in older and more immunocompromised patients and that isolated fever was more common in female patients. It should be noted that they accumulated their cases from their role as a reference laboratory wherein they tested 74,202 serum samples for Q fever over this time period. It is thus difficult to know if some of their observations such as hepatitis occurs in younger patients and pneumonia in older patients are correct and not a result of testing bias. There is emerging evidence that the dominant manifestation of acute Q fever has at least a geographic basis. In Nova Scotia, Canada, most cases of acute Q fever are pneumonia.[52] Nearly all of these were due to exposure to infected cats. In the Basque region of Spain, the manifestations of Q fever were almost equally hepatitis and pneumonia.[53] In the south of Spain in a study of 231 cases between 1985 and 1999, 231 (90%) presented with fever and 148 (64%) had hepatitis.[54]

There are two potential explanations for the different manifestations of the disease according to geographical location. One is that different strains of *C. burnetii* result in different disease manifestations. The French group has taken epidemiology to the molecular level by typing 173 isolates of *C. burnetii* collected from around the world. They used multi-spacer sequencing of the entire genome to type the organisms.[55] They were able to resolve all of the isolates into three monophyletic types. Group 1 consisted of 13 sequence types with isolates from France, Russia, Spain, Kyrgystan, Nambia, Kazakhstan, Ukraine, Uzbekistan and United States. There were two subgroups in monophyletic type 1 with 36 isolates from eight sequence types in the first and 39 isolates from five sequence types in the second. Group 2 isolates were from France, Germany, Switzerland, Romania, Italy, Greece, Austria, Slovakia, United States, Russia, Africa, Kazakhstan, Uzbekistan, Mongolia and Japan. It was divided into four subgroups. Monophyletic group 3 consisted of only one sequence type with 10 isolates. Seven of these were from Nova Scotia patients, two from France and one from United States.

The other potential explanation for the differing manifestations of acute Q fever in different geographic regions is that the route of infection might have a role to play. Thus ingestion of contaminated milk might result in hepatitis and inhalation of contaminated aerosols could result in pneumonia. There is some experimental evidence that supports this.[56]

The French group made two other major contributions to the epidemiology of Q fever. The predominance of Q fever among males has long been accepted as due to the fact that predominately male occupations (animal herders, abattoir workers, animal shearers, farmers, etc.) were associated with the traditional risk factors for Q fever. It is noteworthy that this gender bias is not evident among Q fever cases in children.[57] When C57/BL6 mice were injected with *C. burnetii,* bacterial load and granuloma numbers were lower in females than in males.[58] However, oophorectomized mice had the same bacterial load as males. Treatment of oophorectomized mice with 17 beta-estradiol reduced the bacterial load and number of granulomas in these mice. These findings led the authors to conclude that female sex steroids are protective against Q fever.

The French group was able to demonstrate that *C. burnetii* could be spread at least 11 km downwind from a contaminated source.[59,60] This finding has been confirmed by a group from the United Kingdom.[61]

Two other major contributions of the WHO Rickettsial Laboratory in Marseille to the study of the epidemiology of Q fever are the development of the shell vial technique for the isolation of *C. burnetii* from a variety of biological samples[62] and development of a polymerase chain reaction for the application of *C. burnetii* DNA thereby allowing for molecular diagnosis of this infection.[63]

5.1.5. United Kingdom. In 1958 Marmion and Stoker[64] noted that there had been 104 cases of Q fever in humans in Great Britain and that the peak months for infection were April, May, June, July and October. This group also noted that 6.9% of herds of cattle tested in England, 2% in Wales and 0.8% in Scotland were infected with *C. burnetii* as judged by isolation of the organism. In 1956 Marmion and Stoker[65] described the role of contaminated fomites in the spread of Q fever when they investigated an outbreak of Q fever among 10 amateur actors who had performed a play at a village church at Easter 1949. A shepherd who had a role in the play came to rehearsals with his dog and he was still dressed in his working clothes. They were able to isolate *C. burnetii* from one specimen of dust collected from the clothing of the shepherd.

Palmer and Young[66] reported on all cases of Q fever endocarditis in England and Scotland between 1975 and 1981. They noted that Q fever endocarditis accounted for 11% (92/839) of the cases of Q fever and for 3% of all cases of endocarditis during this period.

Early on in the study of Q fever in Great Britain, Stoker and Marmion[67] noted that man was the only animal species

to regularly develop illness when infected with *C. burnetii*. They also noted that while *C. burnetii* has been isolated from 22 species of ticks, transovarial passage was achieved in only 4 species suggesting that *C. burnetii* cannot be maintained indefinitely in successive generations of its arthropod host without re-infection from another host.

From 1984 to 1994, 1,117 cases of Q fever were reported from England and Wales.[68] The mean age was 45 years and 75% were men. Contact with animals, mainly cattle and sheep, was reported in 60% of cases. Occupationally acquired infection was reported for 24 cases and included abattoir workers, farmers, veterinary surgeons, hide handlers and butchers. Forty-seven percent presented with respiratory symptoms, 7% with heart disease and 5% with hepatitis. Seven percent reported traveling abroad before becoming ill.

Recent events in the epidemiology of Q fever in Great Britain include a July 2002 outbreak at a packing plant in Newport Dock in South Wales.[69] In this outbreak 59/214 (27.5%) of the employees were infected. The plant manufactured corrugated cardboard products and no source for the Q fever was found. From July 9 to July 24, 2006, 24 persons who worked at a meat processing company in Bridge of Allan, Stirlingshire, Scotland, developed Q fever.[70]

5.1.6. Q Fever in Other Countries. *Coxiella burnetii* has been an extraordinarily successful pathogen. By 1955 it was found in 51 countries on five continents.[71] Indeed by the 1990s New Zealand was one of the few countries that were free from *C. burnetii* infection.[72] Arricau-Bouvery and Rodolakis[72] reviewed 18 major outbreaks of Q fever from 11 countries that had been reported from 1999 to 2004. The countries involved were France, five outbreaks; Australia, two outbreaks; Italy, two outbreaks; Japan, two outbreaks; Bosnia, Israel, French Guiana, Canada (province of Newfoundland and Labrador), The Netherlands, Germany and Kenya, one each. Goats were the implicated source in nine, goat manure three, travel associated two, dogs and cats one, wild reservoir one and unknown one.

5.2. Q Fever, the Military and Bioterrorism – Including Contagiousness

Christopher et al.[73] noted that Q fever may have been a significant factor during the American Civil war. During World War II outbreaks of Q fever occurred among American and British soldiers in Greece and Italy. American soldiers in Panama also developed Q fever. Some members of the Axis troops serving in Bulgaria, Greece, Crimea, Bulgaria and the Ukraine developed Q fever. Seven outbreaks of Q fever occurred among US troops in the Mediterranean theater resulting in more than 1,000 serologically confirmed cases. Cases were epidemiologically linked to occupancy of barns and two story dwellings where the bottom story served as a barn. After World War II an explosive outbreak occurred among troops in Libya and most recently cases were acquired during operation Desert Storm. In 2003, 3 of 19 cases of severe pneumonia among American Soldiers serving in Iraq were due to *C. burnetii* and later 5 more cases of Q fever infection were documented.[74]

Q fever was diagnosed at four UN stabilization bases in Bosnia and Herzegovina in 1997.[75] There were 26 cases with an overall attack rate of 4.6%. Five soldiers had pneumonia. A sheep farm was located 100 m from the base. Helicopters landing nearby may have generated aerosols and may have been the source of the outbreak.

Coxiella burnetii is a CDC category B bioterrorism agent.[76] Category B agents have the potential for large-scale dissemination but generally cause less illness and death than category A agents.[77] As a biological warfare agent, *C. burnetii* can be easily dispersed as an aerosol with a high infectivity rate and pneumonia as the major manifestation. The possibility that *C. burnetii* could be used as a bioterrorism agent has resulted in renewed interest in the dynamics of aerosol transmission of this microorganism. It has been calculated that a concentration of 100 cells per m^3 and an exposure time of 30–60 min can deposit more than one cell in the lung and hence result in infection.[78]

5.3. Epidemiology of Q Fever at the Community Level

The study of outbreaks using "shoe leather" epidemiology at the community level has given us much of our information about Q fever. The story of Q fever in Nova Scotia, Canada, illustrates this very well. Q fever was first recognized in Nova Scotia in 1979 during a study of atypical pneumonia.[79] Before too long it was evident that Q fever was occurring in small outbreaks involving family units. Empiric observations and a case–control study as well as isolation of *C. burnetii* from parturient cats associated with these outbreaks confirmed the hypothesis that Q fever was associated with exposure to parturient cats and stillborn kittens in this province.[80–83] Surprisingly in 1990 outbreaks of Q fever in Nova Scotia seemed to cease. Sporadic cases continue to occur but it is now rare to see outbreaks associated with infected parturient cats. Outbreaks of Q fever associated with exposure to infected parturient cats also occurred in the neighboring provinces of Prince Edward Island and New Brunswick. There was also such an outbreak in Maine.[84] There is a suggestion that cats may be associated with Q fever in Japan.[85] Many of those who became ill in Nova Scotia following exposure to infected parturient cats had pneumonia. Radiographically this manifested as single- or multiple-rounded opacities. Most of the patients were

moderately to severely ill and required hospitalization. Improvement occurred rapidly following the administration of appropriate antibiotic therapy. Reactive thrombocytosis occurred in about half the patients with platelet counts reaching 1 million per mm^3. There was only one death among about 300 patients with Q fever pneumonia and that patient had concomitant aspiration pneumonia and probably died from gram-negative sepsis. During the time when cat-related outbreaks of Q fever were occurring the incidence of Q fever in NS was very high at 50 cases per million persons per year.

Cases of Q fever in NS were also associated with exposure to rabbits, deer and cattle.[86–91]

In 1995 we noted our first outbreak of Q fever due to exposure to an infected parturient dog; however, dog-associated Q fever seems to be exceedingly rare.[92] In 2006, a case of Q fever pneumonia as a result of contact with infected parturient sheep occurred in Nova Scotia. This person has experienced considerable fatigue following recovery from the pneumonia. In a survey carried out in 1982 only 6.7% of 329 sheep tested had low levels of antibodies to *C. burnetii*.[93] As part of the investigation of the sheep-associated case of Q fever in NS in 2006 blood from 30 ewes was tested and over 50% had antibodies to Q fever. In a study carried out in 1984, 49% of 65 veterinarians and 35% of 96 slaughterhouse workers tested had antibodies to phase II *C. burnetii* using the microimmunofluorescence test.[81] Contrast this with the seroprevalence of 11.8% among 997 blood donors in NS in 1982.[94]

No cases of Q fever had ever been diagnosed in Newfoundland until 1999. At that time an investigation of an outbreak of abortions among goats revealed that 66/179 goat farmers, their family members and farm workers had acute Q fever.[95] About 1 year previously the community of Bonavista imported goats from Ontario, Prince Edward Island and Maine to start a goat-farming co-operative. At the time of the investigation most of the goats were infected. The clinical manifestations were mainly those of a self-limited febrile illness.[95] However, there was a secondary case that was most unusual.

One of the workers had her clothes washed at home; her mother, a 53-year-old lady, underwent coronary artery angioplasty 6 days before the onset of the symptoms of acute Q fever. Within 4 months she demonstrated the serological profile of chronic Q fever.[96]

Q fever is relatively uncommon in Alberta, Canada, but in recent years there have been 3–6 cases per year. The epidemiological associations are with exposure to goats and sheep. Interestingly, urticaria that persists for 6 or so weeks has been a prominent part of the Q fever illness in three patients (Marrie TJ – unpublished observations).

There have been many outbreaks, over the years, which provide additional information about the epidemiology of Q fever. British residents who lived along a road over which farm vehicles traveled developed Q fever as a result of exposure to contaminated straw, manure or dust from farm vehicles.[97] Four hundred and fifteen residents of a Swiss Valley who lived along a road over which sheep traveled to and from mountain pastures developed Q fever.[98] Those who lived in six villages close to the road had high rates of infection, ranging from 11.8 to 35.8% (mean 21.1%), while those who lived in villages off the road had significantly lower rates of infection, range 2.1–6.8% (mean 2.9%). A recent paper by Senn[99] reviews the above outbreak and concludes that techniques of statistical analysis developed for analysis of experiments, for which the usual precautions of randomization ensure independence, are not necessarily appropriate to the analysis of epidemics. An outbreak of Q fever involving 58 persons in Northern Italy was associated with three flocks of sheep that passed through the area between late May and early June.[100] The prevalence of *C. burnetii* antibodies in these flocks ranged from 45 to 53%. A very similar event was attributed as the cause of an outbreak of Q fever in a prison in Italy wherein 65/600 inmates were infected.[101] Yet another outbreak occurred in Northern Italy at a private residential community for the rehabilitation of drug users.[102] Two hundred and thirty-five of the residents developed Q fever in two consecutive outbreaks. Both outbreaks occurred just after lambing. Forty percent of the 828 or so residents were HIV positive. They were able to show that there was no difference in the manifestations of Q fever according to HIV status and there were no relapses or chronic infection.[102]

Increasingly urban residents visit farms for a variety of reasons. Such visits can result in Q fever as described in Southern France.[103]

On May 3 and 4, 2003, people visited a farmers' market in a spa town close to Soest, Germany, where a sheep had lambs.[104] Subsequently 299 cases of Q fever were linked to this event. The mean incubation period was 21 days with an interquartile range of 14–24 days. About 25% of the adult population was infected compared with 5% of the children and 25% required hospitalization.[104] The implicated ewe was seropositive for *C. burnetii.*

As our use of animal products changes so too does the epidemiology of Q fever. A small factory, employing eight persons, in Victoria, Australia, manufactured powdered sheep placenta and fetal tissue for the cosmetics industry. In February 2005 as a result of a request for amniotic fluid the manufacturing process was changed and four employees developed Q fever.[105]

In a town in France, 29 cases of Q fever occurred due to aerosols created by helicopters landing close to a slaughterhouse with uncovered sheep waste.[106] In 1951 Harvey and colleagues[107,108] reported a unique outbreak of Q fever at a

College in Cambridge, England. They attributed the outbreak to straw in a packing case.

Even an activity such as playing poker can result in Q fever if your infected cat gives birth to kittens in the immediate vicinity of the poker game.[109]

Reilley and co-workers[110] reviewed all cases of Q fever that had occurred over 16 years in their hospital catchment area. Their investigations uncovered some old and some new sources for Q fever. Thus eight persons developed Q fever following renovations on former farm buildings. They felt the source was disturbance of contaminated dust during renovation work. Two members of a film crew developed Q fever following exposure to windborne straw and peat.[110] This report is remarkable because of the high rate of neurological complications that ensued in the patients with Q fever.

In the summer of 1981, 29 patients living in Gwent, South Wales, had Q fever.[111] Twenty were diagnosed by routine clinical and laboratory services and nine were discovered by active case finding. The date of onset of symptoms spanned a 1–2-month period. The peak incidence was at the end of June. A case–control study showed an association with one area on the outskirts of Newport. Most patients had no direct contact with farms or farm animals and a common source of infection could not be identified. The most likely explanation of the outbreak was that farm vehicles had disseminated contaminated straw, manure or dust in the area of Newport adjacent to farmland. The last straw episode is one from an outbreak of Q fever in South Wales, United Kingdom, from July 15 through September 30, 2002.[112] Ninety-five of 282 (33.6%) employees of a cardboard manufacturing plant developed Q fever. The offices were undergoing renovation and drilling into contaminated straw board was felt to be the likely source of the outbreak.

One of the largest case series of Q fever was from Bulgaria.[113] Until 1990 there were more than 20 outbreaks per year, some with hundreds of patients. Q fever has been known in Slovakia since 1954 when outbreaks occurred among agricultural workers who contracted infection from sheep imported from Romania.[113] From 1954 to 1980 waves of epizootics and small epidemics appeared in factories processing cotton, wool and hides from Mongolia and China. Since the 1980s only sporadic cases have occurred. Mass vaccination of cattle was carried out in Slovakia in the 1970s.

There have also been outbreaks of Q fever where unusual sources have been postulated with less than clear evidence of a cause-and-effect relationship.[114] One such outbreak involved 16 cases among family members or visitors to a farm in South Kent. A piglet bitten by a rat was nursed in the farmhouse and was handled by a man who was one of those who were infected. However, a sow also aborted within the time frame for the incubation period for Q fever. Also of interest was that the patients were treated with chloramphenicol with a good response. Another unusual outbreak was one that occurred in a hospital kitchen in Israel.[115] Sixteen cases occurred over 42 days among the 41 staff members. No clear source for the infection was found although it was postulated that a contaminated ventilation shaft or contaminated clothing was the source.

The first case of Q fever in Uruguay was described in 1956 in a slaughterhouse worker. Fourteen outbreaks, involving 1,358 suspect clinical cases, were studied between 1975 and 1985. All occurred among workers at meat-processing plants. The incubation period ranged between 13 and 21 days.[116]

A prospective study was designed to determine the occupational risk of Q fever in five English local authority districts.[117] The seroprevalence of Q fever was measured and the cohort followed for 2 years. There were no seroconversions during the first year but two occurred during the second year for an incidence of 813/100,000 per year (95% confidence intervals 98–2,937/100,000 per year). Seroprevalence was higher among the study cohort 105 of 385 versus 43 of 395 (27.2%) compared to 43 of 395 (10.8%) among other UK farm workers ($P < 0.01$). The authors concluded that the risk of Q fever on livestock farms is related to contact with the farm environment rather than any specific animal exposure. They also indicated that the absence of an increasing prevalence with age suggests that exposure may occur as clusters in space and time, i.e., outbreaks.[117]

A prospective surveillance study in Cyprus also adds to our knowledge of the epidemiology of Q fever.[118] Before considering the current study it is worthwhile knowing something about the background of Q fever in Cyprus. It was first identified in this country in 1951[71] and serosurveys in 1968 and 1969 demonstrated that 5% of 547 patients at Nicosia General Hospital had antibodies to *C. burnetii*.[118] From December 1974 to June 1975, 78 British soldiers in Cyprus developed Q fever as a result of exposure to contaminated dust from an abortion epidemic among sheep and goats due to *C. burnetii*.[119] At that time no cases were reported from the local population. In the current study[118] seropositive humans and animals in two villages were identified and then all seronegative individuals were followed for 1 year on a monthly basis. Eighty-one humans and 239 animals from both villages participated in the first phase. Two humans and one goat were identified as having clinical Q fever; however, 44.4% of the humans and 13.8% of the animals were seropositive at the end of 1 year. During the second phase of surveillance, nine human, eight goat and four sheep cases

were serologically confirmed and *C. burnetii* was isolated from three human and two animal samples for an incidence rate among humans of 1.2/100,000 per year.[119] In another study of Q fever in Cyprus, Psaroulaki et al.[120] obtained serum samples from 583 humans, 974 ruminants and collected 141 ticks from animals. They used PCR to detect *C. burnetii* DNA in the ticks, and a geographical information system was used to identify high-risk regions. The seroprevalence for Q fever was 52.7% for humans, 48.2% for goats, 18.9% for sheep and 24% for bovines. Just over 11% of the ticks had *C. burnetii* DNA. Using the geographic information system they identified two high-risk villages. The risk factors for *C. burnetii* infection among humans were age, residence, occupation, use of manure in garden, ownership of animals (especially goats) and the presence of tick infested or aborting animals.

5.4. Q Fever in Animals

As is evident by now Q fever is a zoonosis and direct or indirect contact with infected cattle, sheep, goats, cats and dog or the products of conception of these animals are the primary source of Q fever for humans. Contamination of the environment by fluids from these animals is also an important source of Q fever for man. A full understanding of the epidemiology of Q fever in humans requires knowledge about coxiellosis in animals. *C. burnetii* localizes to the uterus and mammary glands of infected animals[121] Infected cows can shed *C. burnetii* in milk for up to 32 months.[122] Large concentrations of *C. burnetii* are present in the infected placenta, and aerosols are created during parturition.[123] Inhalation of these contaminated aerosols by a susceptible human, results in Q fever.

Coxiella burnetii infection has caused outbreaks of abortion in goats[121,122] and sheep.[123,124,125] Sanford et al.[123] described abortions that occurred in five goat herds that were exposed to three goats from another herd that kidded prematurely during a fair. All of the goats were housed in the same barn. Abortions began in the exposed herds 21 days after the exposure and affected 20–46% of the pregnant animals in each herd. Immunohistochemistry methods have shown that organisms are present on both surfaces of the chorioallantoic membrane of the affected placenta.[126] The placentas of infected sheep can contain 10^9 guinea pig infective doses of *C. burnetii* per gram of tissue.[127,128]

The stillbirth rate among infected cats is about 70% compared with the usual rate of 10% for uninfected cats.[129]

Dairy cows are important in the spread of Q fever, while beef cows are rarely infected.[130] Once *C. burnetii* is introduced into a herd rapid spread occurs; so that 80% of the cows are positive within a few months.[131] Infected wildlife may be important in infecting cattle since in a large portion of newly infected herds a source of infection is not found.[131,132]

Hares and rabbits in many areas have the highest rate of infection among wild animals.[132] In a study in Oxfordshire, Webster et al.[133] found that 7–53% of wild brown rats (*Rattus)* were seropositive for *C. burnetii.* These workers postulated that since cats are frequent predators of rats this explains why cats become infected.

Infected ticks are probably most important in keeping the whole cycle of *C. burnetii* infection going.[67,118] Infected ticks have been found on rabbits, goats, cattle, sheep and many other animals.[67,118] It is likely that birds are also important in the spread of *C. burnetii* by transporting infected ticks from one area to another. Birds may also be involved directly in the spread of Q fever to man as evidenced by a recent outbreak of Q fever among family members in Southern France due to aerosols from *C. burnetii* infected pigeon feces.[134]

In some countries infection among domestic or wild animals results in considerable infection among humans in contact with these animals, whereas in other areas little if any transmission to man occurs.[64]

Ewes shed *C. burnetii* in vaginal mucus and feces more often and for a longer time than cows however cows and goats shed the organism for a longer time in milk.[135] Berri et al.[136] noted two abortions associated with *C. burnetii* infection among a group of 34 pregnant ewes managed by the experimental Infectious Pathology and Immunology unit of the institute in Tours-Nouzilly, France. They treated the ewes with oxytetracycline to prevent spread of the infection and further abortions. However 5 abortions occurred in January and March 1999 in three groups of ewes and 24 of the ewes shed *C. burnetii* in vaginal secretions. Twelve percent of 113 lambs born in 1999 were seropositive for *C. burnetii* and half of the seropositive lambs were born to either seropositive ewes or dams that excreted the pathogen in their vaginal tracts. However in 2000 all 150 lambs were seronegative.[136]

In goats active *C. burnetii* infection seemed to be limited to two pregnancy cycles.[137] Berri et al.[136] found that following a first kidding season when only 57% of 56 pregnant goats had a normal delivery in the second season 87% had a normal delivery. They noted that goats shed *C. burnetii* in milk for 3 months.[136,138] The Berri group[139] also noted that goat manure used as garden fertilizer can be a source for Q fever in humans.

In cattle metritis is a unique manifestation of this infection.[140] We were able to associate an outbreak of Q fever in a town with metritis due to this organism in a cat.

6. Routes of Transmission

6.1. Inhalation of Contaminated Aerosols

Experimental and epidemiological evidence lead to the conclusion that this is the major mechanism whereby Q fever is transmitted to man.[59–61,141–145] Volunteers who inhaled one infectious dose had an incubation period of 16 days, whereas those who were exposed to 1,500 infectious doses had an incubation period of 10 days.[141] Indirect exposure to contaminated material may also lead to Q fever such as when contaminated clothing from the Rocky Mountain laboratory in Montana led to cases of Q fever among laundry workers.[142] Another unusual outbreak of Q fever occurred among factor workers who were handling contaminated imported cotton.[143] There have been several outbreaks of Q fever in research institutions when infected pregnant animals, especially sheep, were transported through the building to the laboratory.[144–147] Carefully conducted studies have documented that high winds can result in infection with *C. burnetii* up to 11 miles (18.3 km) distant from the point source.[59–61] A petting zoo in two shopping malls in Quebec, Canada [13 goats and 5 sheep on exhibit between March 25 and April 3, 1999] was the source of 95 cases of Q fever.[148]

6.2. Oral Route

There is a suggestion from epidemiological studies that ingestion of contaminated milk is a risk factor for Q fever infection.[149–153] However, evidence from experiments where contaminated milk was fed to volunteers is contradictory.[151–153] In a case–control study Hatchette and co-workers[154] found that ingestion of pasteurized cheese and tobacco smoking were both risk factors for acquisition of Q fever during an outbreak of Q fever on caprine cooperative farms in Newfoundland.

We feel that the route of infection may explain the difference in the manifestations of Q fever in some countries, e.g., pneumonia in Nova Scotia, Canada versus hepatitis in Marseille, France.[155] We used five different strains of *C. burnetii* to infect mice via the intraperitoneal or intranasal route. Those infected intranasally developed pneumonia only, while those infected intraperitoneally developed hepatitis, splenomegaly and pneumonia. Bronchiolar changes were seen only in mice inoculated intranasally.[155] These data have been reproduced in a guinea pig model of Q fever.[156]

6.3. Percutaneous Route

Crushing an infected tick between the fingers has resulted in Q fever[157] and blood transfusion.[158]

6.4. Vertical Transmission

This occurs rarely but increased surveillance may reveal additional cases of vertical transmission.[159–161] In the town of Martigues in Southern France Q fever complicated at least 1 in 540 pregnancies.[161]

6.5. Person-to-Person Transmission

Despite the fact that pneumonia (and hence the potential for spread via aerosols) is a common manifestation of Q fever in man there have been only a few cases of person-to-person transmission.[162–164] The intracellular location of the organism is likely the explanation for this. There are two reports of transmission of Q fever to attendants during autopsies,[162,163] and one report of transmission of infection from a patient to hospital staff.[164] Since infected parturient cats, sheep and cattle readily spread infection to man – one wonders why more cases of Q fever do not develop in obstetrical staff who assist at the delivery of infected pregnant women. We have observed two pregnant women with large numbers of *C. burnetii* in the placenta yet Q fever did not develop in any of the obstetrical attendants, however Stein and Raoult[160] did document Q fever in an obstetrician who attended the delivery of a woman with Q fever during pregnancy. Nevertheless person-to-person transmission is so infrequent that patients with Q fever are not isolated when they require admission to hospital for treatment.

6.6. Sexual Transmission

Sexual transmission of *C. burnetii* has been demonstrated in mice[165] and viable *C. burnetii* cells have been demonstrated in bull semen.[166] There has been speculation that Q fever can be sexually transmitted among humans.[167] Milazzo et al.[168] reported the case of a 53-year-old man who developed orchitis as a complication of Q fever. The orchitis had its onset 3 days after he had intercourse with his spouse (29 days after onset of his illness). Fifteen days later his spouse developed Q fever. *C. burnetii* DNA was identified by PCR in the semen of the index case 4 and 15 months after onset of the acute illness. These data indicate that *C. burnetii* can be transmitted sexually in humans.

7. Biological Characteristics of the Organism: Pathogenesis and Immunity

The interaction of *C. burnetii* with the human host is a fascinating story in bacterial pathogenesis that is still incompletely understood.[15,169–178] The T-cell-dependent immune control of *C. burnetii* does not lead to its eradication and thus immunosuppression can induce relapse of infection in apparently cured patients or laboratory animals.[46,48] Recently, Harris et al.[46] showed that *C. burnetii* DNA could be demonstrated by PCR in circulating monocytes and bone marrow months or years after acute Q fever. These findings in conjunction with a certain host response could explain why chronic Q fever and persistent fatigue syndrome occur.[42–43] Another key observation which relates to the immune response to *C. burnetii* is that very few organisms and a strong cellular response are seen in patients with acute Q fever, while many organisms and a moderate cellular response are seen in patients with chronic Q fever.[177]

8. Patterns of Host Response

Q fever can be broadly divided into two major categories, acute and chronic. Acute Q fever is easily managed and most cases are self-limited and indeed are not recognized either by the patient or by the physician. Chronic Q fever is a serious condition with considerable morbidity and mortality. Most cases of chronic Q fever are endocarditis. The clinical manifestations of Q fever are summarized briefly here but are referenced so that interested readers can read more.

8.1. Acute Q Fever

There are several manifestations of acute Q fever:

8.1.1. A Self-Limited Febrile Illness. This is the most common manifestation of acute Q fever. Most individuals who fall into this category do not know that they have had Q fever. The illness is considered to be one of the many non-specific illnesses that one suffers from during life. Occasionally fever is prolonged and necessitates hospitalization and a fever of unknown origin workup.[181,182]

8.1.2. Pneumonia. This manifestation of acute Q fever differs according to geographic site. The manifestations of pneumonia are the same as for any other pathogen – fever, cough (usually non-productive) and in severe cases shortness of breath. The radiographic manifestations are often distinctive and show single- or multiple-rounded opacities.[183–189]

8.1.3. Hepatitis. In most instances the hepatitis is manifested as elevated liver enzymes. Occasionally jaundice is the presenting manifestation of Q fever. Prolonged fever is not unusual in patients with Q fever and hepatitis. This fever frequently does not respond to antibiotic therapy and a short course of corticosteroid therapy is necessary.[190–199]

8.1.4. Miscellaneous Manifestations. At times manifestations other than the three mentioned above dominate the clinical picture. Thus the presentation could be that of central nervous system infection; peri or myocarditis; lymphadenopathy, splenic rupture or orchitis.[199–209]

8.1.5. Q Fever in Immunocompromised Host. Q fever can occur in the immunocompromised host. Most clearly documented to date is infection in patients with human immunodeficiency virus infection. Recently there has been a case report of Q fever pneumonia in a patient with a renal transplant.[210–215]

8.2. Chronic Q Fever

There are six main presentations of chronic Q fever

1. Endocarditis – about 90% of the cases of chronic Q fever are endocarditis. The diagnosis and management of this condition are difficult. The current therapeutic regimen of choice is doxycyline plus hydroxychloroquine.[216–240]
2. Endovascular infection.[241]
3. Osteomyelitis.[242]
4. Q fever in pregnancy.[243–246]
5. Hepatitis – most of the cases of hepatitis due to *C. burnetii* are manifestations of acute Q fever. On occasion, however, hepatitis can be chronic.[51]
6. Miscellaneous manifestations – these include livedo reticularis, chronic thrombocytopenia and mixed cryoglobulinemia.[51]

9. Control and Prevention

9.1. General Concepts

Good animal husbandry practices are the primary methods in preventing widespread contamination of the environment by *C. burnetii*. Prevention of zoonotic spread is best accomplished by isolating aborting animals for up to 14 days, raising feed bunks to prevent contamination of feed by excreta, destroying aborted materials by burning and burying fetal membranes and stillborn animals and by wearing masks and gloves when handling aborted materials.[247,248] Treatment of infected animals with tetracycline compounds may also have a role in limiting the spread of *C. burnetii* infection. There are insufficient data on vaccination of animals to know whether or not this strategy has a role in limiting spread of Q fever.

Only seronegative pregnant animals should be brought into the facilities where research will be done. In addition only seronegative animals should be used in petting zoos.

9.2. Chemotherapy and Prophylaxis

The major role for prophylaxis is in the event of widespread contamination such as from a bioterrorism event. The European guidelines recommend prophylaxis within 1 week of exposure with doxycycline 100 mg bid po.[247] Other choices are erythromycin, clarithromycin or roxithromycin. The treatment of acute and chronic Q fever is outlined in Section 8.

9.3. Immunization

Coxiella burnetii vaccines are not generally available. However, Q vax (heat-inactivated phase 1 cells) has been used very successfully in Australia. Potential vaccines should be antibody and skin test negative for *C. burnetii* to avoid adverse reactions. Sterile abscess at the site of injection is the most common and most severe adverse reaction. High-risk groups (in endemic areas) such as abattoir workers, veterinarians, laboratory workers who isolate *C. burnetii* should be immunized.[248,249] While a Q fever vaccine is not available commercially in the United States, such a vaccine is available from the Centers for Disease Control.[248] Marmion has recently reviewed the history of Q fever vaccine in Australia and briefly discussed chemovaccine (used in Eastern Europe) and the search for newer vaccines.[250]

10. Unresolved Issues

There are many unresolved issues regarding Q fever. Why does the epidemiology vary from country to country and even within a country? Why does the rate of Q fever post-fatigue syndrome vary from country to country? Other unresolved issues include policies on surveillance of animals and regulation of sale of seropostitive animals.

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12. Suggested Readings

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CHAPTER 31

Rocky Mountain Spotted Fever

Aaron Milstone and J. Stephen Dumler

1. Introduction

Rocky Mountain spotted fever is an acute febrile illness transmitted to man by ticks infected with *Rickettsia rickettsii*. Usually sudden in onset, it is characterized by chills, headache, and fever lasting 2 or more weeks. A characteristic rash appears on the extremities on about the 4th febrile day and spreads to the trunk. The exanthem and other anatomical manifestations result from focal areas of vasculitis and perivascular inflammation scattered throughout the body. Central nervous system (CNS) manifestations of delirium and coma as well as shock and renal failure occur in the severely ill. Serum antibodies to specific rickettsial antigens appear during the 2nd and 3rd weeks of illness. The tetracyclines are highly effective therapeutically.

2. Historical Background

Idaho physicians described a form of "black measles" in the Snake River Valley as early as 1873. In 1899, Maxcy described a febrile illness with delirium and a blotchy-skin, red–purple–black rash that appeared first on the ankles, wrists, and forehead, with rapid general body spread. This was the "spotted fever of Idaho." It was noted to be sporadic and more common in the spring, and local opinion attributed it to the drinking of water from melted snow or inhalation of sawdust.[1] Dr. Earl Strain, a practicing physician in Great Falls, Montana, first suspected the role of ticks, having noted a relationship between a death and a history of a tick bite. Wilson and Chowning[2] concurred with this relationship and concluded that the disease was transmitted by a local wood tick and the ground squirrel (gopher) as a possible reservoir.

In concert with the prevailing opinions of the times regarding tick-transmitted infections, they proposed *Piroplasma hominis*, an erythrocyte parasite, as the causative agent. In a series of brilliant studies carried out from 1906 to 1909, usually during the spring and summer months, Howard Taylor Ricketts successfully transmitted the disease to guinea pigs and monkeys by inoculation of blood from patients with spotted fever, incriminated the wood tick, *Dermacentor andersoni*, as vector by feeding experiments on animals and demonstrated the occurrence of naturally infected ticks.[3] He observed bacteria-like bodies in smears prepared from tick tissues and showed the transovarial transmission of infection to offspring of infected female ticks. Ticks were shown to be infected throughout their life span. Studies of immunity showed that blood from animals that survived the infection protected other animals if blood was administered several days before infection.[4,5] Wilder and Ricketts showed through cross-immunity studies that spotted fever and typhus were distinct entities. Ironically, the Montana legislature in 1909 allocated the sum of $6,000 for Rickett's research studies, which the board of examiners failed to appropriate.[1] In July 1909, Ricketts proceeded to Mexico to study typhus fever; there, his life was cut short by this disease, which is closely related to Rocky Mountain spotted fever. In 1916, Dr. da Rocha Lima, another pioneer in this field, gave the name *Rickettsia* to those agents that cause the typhus and spotted fever disorders. An independent study conducted by McCalla and Brereton of Boise, Idaho, in 1905, unknown to Ricketts, showed that the bite of a tick removed from one of their patients transmitted spotted fever to two

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This chapter is dedicated to the profound contributions of the late Theodore E. Woodward, M.D. toward understanding and management of human rickettsial infections and retains significant content from the 3rd Edition contributed by him.

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human subjects—a healthy prisoner and a woman—in whom a moderate and a mild attack of Rocky Mountain spotted fever occurred. This work was not published until 1908.[6]

Wolbach[7] was the first to detail the microscopic lesions of Rocky Mountain spotted fever with descriptions of the classic focal lesions of blood vessels. He distinguished between pathogenic and nonpathogenic organisms in ticks and demonstrated the intranuclear multiplication of rickettsiae in tick tissues.

Much knowledge was added by Spencer and Parker,[8] who showed that guinea pigs could be infected readily by intraperitoneal injection of macerated tick tissue. Ticks obtained in the Bitter Root Valley during early spring failed to cause illness in guinea pigs. Ticks fed on infected goats killed inoculated animals. These workers demonstrated "reactivation" by showing that unfed ticks immunized animals despite their inability to cause demonstrable infection and that virulence could be revived by providing ticks a blood meal (see Section 4). Also, phenolyzed tick juice effectively afforded protection in guinea pigs. Vaccinated humans developed antibodies, and their sera protected guinea pigs from experimental infection. Inactivated infected tick-tissue vaccine for man became a reality.[8]

Cox[9] provided an additional milestone by showing that rickettsiae propagated well in chick-embryo yolk sacs, particularly after death of the embryo. The yolk sac vaccine was simpler to produce and gave fewer reactions.

Therapeutically, immune serum did not prove to be really effective in patients with Rocky Mountain spotted fever. This is principally because rickettsiae are localized intracellularly at the time when the clinical diagnosis becomes apparent, on about the 5th or 6th day. The first effective and specific treatment was para-aminobenzoic acid, which did reduce mortality and morbidity, but it proved awkward to use.[10]

In 1948, first with chloramphenicol and later with the tetracyclines, specific treatment was placed on a firmer basis, and therapy for Rocky Mountain spotted fever became readily available.[11]

It is quite likely that Rocky Mountain spotted fever was prevalent in the eastern Atlantic states before its reported recognition in 1930. Most of the cases in the East probably masqueraded as endemic typhus or were called Brill's disease. Pinkerton and Maxcy[12] reported a case in Charlottesville, VA, that was undoubtedly Rocky Mountain spotted fever. Dyer et al.[13] noted, in 1930, that patients whose illness occurred in the spring and summer along the Eastern Seaboard states closely resembled Rocky Mountain spotted fever. One of these patients was recognized in Maryland by Maurice C. Pincoffs;[14] Dr. Pincoffs's case was published by Dyer et al.[13] In 1901, Sir William Osler diagnosed a patient with typhus at the Johns Hopkins Hospital in Baltimore, MD. The patient died and the autopsy performed by William Henry Welch revealed findings consistent with the emerging pathological characteristics of typhus fever. Ninety years later, the autopsy materials were retested using an immunohistological technique and were clearly demonstrated to contain *R. rickettsi*, proof that Rocky Mountain spotted fever existed in the eastern United States concurrent with its discovery in the West.[15]

3. Methodology

3.1. Sources of Mortality Data

The mortality from Rocky Mountain spotted fever has been variable, especially after the introduction of antibiotics, so that mortality data are not a reliable indicator of incidence. Prior to specific antibiotic usage, the overall mortality rate annually averaged about 20% for all ages. In the mountain and eastern states, where occupational pursuits exposed adults to infected ticks, fatality exceeded 50% in those persons aged 40 or older. It was appreciably lower in children and young adults. Two developments have drastically improved treatment, reduced fatality, and shortened the clinical course of infection. The broad-spectrum antibiotics, first chloramphenicol and later the tetracyclines, were introduced in 1948. When they were given early during the course of Rocky Mountain spotted fever, when the rash was noted, usually on the 3rd–6th day of illness, recovery ensued rapidly. Of equal importance was the elucidation of the pathophysiological abnormalities responsible for the severe manifestations and complications that occur during the 2nd febrile week. Properly applied supportive measures and antibiotic treatment led to recovery in patients who did not come under medical care until late in the illness. Despite these advances in supportive care in managing the late sequelae of late-stage Rocky Mountain spotted fever, a fatal outcome is strongly associated with a delay in diagnosis and therapy.[16,17]

3.2. Sources of Morbidity Data

Rocky Mountain spotted fever is a reportable disease. In patients whose illness is not detected early and treated properly, serious sequelae and a long hospitalization often follow. The Centers for Disease Control and Prevention (CDC) publish the number of cases that occur weekly in various states, previousl under the category of typhus fever ("Tick-borne RMSF"), and now simply as "Rocky Mountain spotted fever". Criteria for diagnosis are somewhat reliable, since

confirmatory serological tests, including the indirect fluorescent antibody (IFA) test or the complement-fixation (CF) reaction, and identification of the *Rickettsia* in skin biopsy by immunohistology are generally available. Some authorities suspect that the data could be confounded by the inclusion of ehrlichiosis and anaplasmosis, or other milder spotted fever group rickettsioses.

3.3. Laboratory Diagnosis

Several types of confirmatory laboratory tests are useful for diagnosis of Rocky Mountain spotted fever: (1) serological tests to detect the presence and increase of specific antibodies in the patient's blood during convalescence;[18] (2) isolation and identification of *R. rickettsii* from blood or tissues; (3) identification of the agent in skin, tissues, or blood by the use of labeled antibodies; and (4) identification of rickettsial nucleic acids in the blood or tissues of infected patients by polymerase chain reaction (PCR) amplification.[19] Since no test is 100% sensitive, a negative laboratory result should not dissuade from institution or continuation of effective therapy. In particular, reliance on serological results during the acute illness is hazardous, and these tests are most useful for confirmation of infection in convalescence. Examination of a peripheral blood smear is not useful for the diagnosis of Rocky Mountain spotted fever.

The CDC defines a confirmed case of Rocky Mountain spotted fever by (1) a fourfold change in antibody titer to *Rickettsia rickettsii* antigen in paired acute and convalescent serum samples, or (2) a positive PCR assay, or (3) immunostaining of antigen in a skin biopsy or autopsy sample, or (4) isolation and culture of *R. rickettsii* from a clinical specimen.[20] The CDC defines laboratory supportive evidence as a clinically compatible case with serologic evidence of elevated lgG or lgM antibody reactive with *R. rickettsii* antigen by IFA, enzyme-linked immunosorbent assay (ELISA), dot-ELISA, or latex agglutination.

3.3.1. Isolation and Identification of *R. rickettsii*. If isolation is attempted, blood should be obtained from the febrile patient prior to antibiotic treatment. Usually, the mononuclear cells are harvested from peripheral blood and used as an inoculum in a centrifugation-assisted shell vial method.[21] Many cell types are susceptible to rickettsial infection, including cells often used in standard virology laboratories, such as Vero cells (African green monkey kidney), L929 mouse fibroblasts, and human embryonal lung (HEL) fibroblast cells, among others. Coupled with the early detection of rickettsial antigen in shell vials by immunofluorescence with species-specific monoclonal antibodies, the diagnosis may be established within as early as 48 h. Isolation in guinea pigs by intraperitoneal inoculation or in chicken embryo yolk sacs is a cumbersome, expensive method rarely used for clinical diagnosis.

3.3.1a. Identification of R. rickettsii *in Tissues.* Immunohistologic techniques, including immunofluorescence and immunoenzymatic methods, are useful for detection of *R. rickettsii* in tissues of patients.[22–24] The most useful specimen is a punch biopsy of a petechial skin lesion. However, the rickettsial infection may be extremely focal, and thus multiple sections must be examined to maximize sensitivity, which averages approximately 70%. The specificity is excellent. Although rickettsia have been observed late after institution of antibiotic therapy, the best opportunity to visualize the organisms by these methods is before initiating treatment, as detection diminishes rapidly thereafter. It must be remembered that a negative result does not exclude Rocky Mountain spotted fever, and thus in suspected cases, therapy should be continued to assure a favorable outcome. The method is useful on both frozen sections and formalin-fixed, paraffin-embedded tissues.[22,25]

3.3.1b. Identification of Rickettsial Nucleic Acids in Clinical Samples. Given the low titer of rickettsiae that are present in peripheral blood of infected patients even at peak rickettsemia, the highly sensitive PCR technique for amplification of rickettsial nucleic acids has been proposed as a specific diagnostic method. PCR can detect DNA in a sample with 5–10 *Rickettsia*. Several target genes can be amplified, including 16S ribosomal RNA, *R. rickettsii* outer-membrane protein (OmpA), a gene encoding the genus-common 17-kDa lipoprotein, and the citrate synthase (*gltA*) gene. The best described is amplification of the 17-kDa antigen gene, and several cases where PCR has allowed a rapid, early diagnosis have been reported.[19] The specificity is dependent on the design of the oligonucleotide primers, but is usually very high. However, the PCR method is no more sensitive than immunohistologic demonstration in skin biopsies,[19] because of the relative lack of rickettsiae in blood at any time. Rickettsial DNA could be best detected in skin biopsies, since the *Rickettsia* are plentiful in sites of vasculitis.[26] DNA amplication is highly specific, but can be confirmed by sequencing to eliminate the possibility of identifying another spotted fever group *Rickettsia*.

3.3.2. Serological Tests. To be useful, serological tests require at minimum two and preferably three serum samples: during the 1st, 2nd, and 4th–6th weeks of illness.[27] Some serological tests such as the latex agglutination and complement-fixation tests are most useful for detection of IgM and for confirmation early after infection and are relatively unavailable now; however, the IFA test, enzyme-linked immunosorbent assay (ELISA), and solid-phase enzyme immunoassay tests (dipsticks)[28] may be useful for detection

of IgG, IgM, or both.[29] Antibodies of the IgG type persist for months to years after the acute infection, and thus tests that detect these immunoglobulins are also useful for seroepidemiological investigations. Typically, a serological diagnosis is rendered when a patient with a consistent illness develops a fourfold rise in antibody titer in convalescence or has a minimum titer that is technique dependent. No serological test has been shown to be highly sensitive during the first 2 weeks of illness, and thus these tests must be considered as confirmatory in convalescence only. No therapeutic decision should be made based on the results of a negative antibody test during acute infection.

3.3.2a. Weil–Felix Reaction. Strains of *Proteus* OX-19 and OX-2 are agglutinated by sera of patients with Rocky Mountain spotted fever. These agglutination tests are now considered to be of historic importance only. The poor sensitivity and very low specificity make these reactions poor predictors of the diagnosis, given the relatively low prevalence of the disease in some geographic regions. Their main advantages are low expense and the ability to test samples under field conditions.

3.3.2b. Indirect Fluorescent Antibody and Microimmunofluorescent Tests (MIF). The standard for rickettsial serological diagnosis is the IFA or MIF test. This test utilizes whole, tissue-culture-propagated rickettsiae, and thus is approximately 99% sensitive.[30,31] In addition, the test has a high degree of specificity for antibodies reactive with spotted fever group rickettsiae; it is rapid and highly sensitive. Some cross-reactivity with typhus group rickettsiae is occasionally seen. Moreover, the test can be used to determine IgG or IgM responses to differentiate recent infection from remote disease. The high sensitivity and ability to distinguish among immunoglobulin classes also makes this test useful for seroepidemiological investigation.

3.3.2c. Other Serological Tests. Because of the lack of a rapid, specific, and sensitive test to replace the outdated Weil–Felix reaction, the latex agglutination test was developed.[32] This serological test uses highly purified rickettsial antigens coated on latex beads, may be rapidly performed within minutes, and is simple to perform and interpret. The test is very sensitive and specific and detects predominantly IgM antibodies useful for early serological confirmation in local hospital and public health laboratories. Another recent addition combines the exquisite sensitivity of enzyme immunoassay methods with the ease of rapid dipstick testing.[28] These tests generally have a high degree of sensitivity and specificity, and some are commercially available.

A recurring problem with the serological diagnosis of species in the genus *Rickettsia* is the lack of specificity to the species level. Thus, with a reactive serological test by any of the above methods, it is impossible to distinguish among agents of the spotted fever group rickettsiae and occasionally it is impossible to exclude typhus group rickettsiae as the causative agent of the disease. The development of a highly sensitive and specific ELISA for Rocky Mountain spotted fever for serological testing, coupled with the use of species-specific monoclonal antibodies that are blocked from attachment by the human antibodies in the test sera, provides a serological tool capable of rendering a diagnosis at the species level.[33] The other serological tests, including the microagglutination test (MA), the indirect hemagglutination test (IHA), and other serological tests such as complement fixation, are no longer used in clinical circumstances.

4. Biological Characteristics of the Organism

R. rickettsii, which possesses the general properties of other rickettsiae, is an obligate intracellular, coccobacillary bacterium. They are minute, averaging 0.6–1.2 μm, and stain purple with Giemsa, light blue with Castenada, red with Machiavello, and bright red with Giménez techniques.[18] These rickettsiae grow in the cytoplasm and nuclei of infected cells of ticks, mammals, and embryonated eggs. Electron micrographs reveal a gram-negative-like cell envelope containing chromosomal material and ribosomes. In some preparations, a microcapsular "slime" layer may be detected. They appear to divide by binary fission.[11,34]

Biochemically, *R. rickettsii* simulate other rickettsiae in chemical composition, metabolic consistency, and nutritional requirements. They contain both RNA and DNA and oxidize certain intermediates of the Krebs cycle.[34,35]

The agent is relatively labile and readily inactivated by heat and chemical agents. Suspensions of infected tissues are inactivated within 24 h by 0.1% formaldehyde or 0.5% phenol. They remain viable for long periods of time when stored at −70°C. When carefully lyophilized from buffered sucrose–glutamate media and stored at 5°C, they remain viable for years.[11]

The antigenic and molecular constituents form the basis for understanding the mechanisms of rickettsial attachment, invasion, and intracellular multiplication. Although *R. rickettsii* has abundant lipopolysaccharide (LPS) demonstrable by chemical and immunoblotting methods, this component contributes little to pathogenesis.[36] In fact, it is likely that the LPS accounts for a large proportion of genus- and group-common antigenicity. By immunoblot analysis, two major immunodominant proteins are evident: a 190-kDa outer-membrane protein (OmpA) and a 120-kDa outer-membrane protein (OmpB).[37] The genes encoding these proteins have been cloned and considerable study has

revealed likely functions for both. OmpA is the putative rickettsial adherence ligand[38] and OmpB functions as both a structural protein and an adhesin.[37,39] Recombinant OmpA has been successfully employed as a protective vaccine in challenged guinea pigs and mice.[40,41] Mutants lacking normal processing of OmpB lack in vitro correlates of virulence.[37]

After rickettsiae attach to a cellular ligand, presumably Ku70 that is induced for surface expression with rickettsial adhesion via OmpB or OmpA,[38,39,42] they gain access to a transiently formed vacuole from which they rapidly escape. Once in the cytoplasm, *R. rickettsii* influences the polymerization of intracellular F-actin, in a manner different from that observed with *Shigella* or *Listeria*.[43–45] This polymerization rapidly mobilizes the organisms and enables cell escape and cell–cell transfer.

R. rickettsii exists in ticks in a nonvirulent form that can be reactivated to a virulent infection-producing phase with a fresh blood meal or when incubated at 37°C for 24 h. This phenomenon probably accounts for the failure of infected ticks, after overwintering, to transmit illness to humans in the early spring until they have attached and fed for at least several hours. Later, during spring and summer, when natural reactivation has presumably occurred, ticks may infect humans after shorter exposure.[11,34]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

The annual number of patients with Rocky Mountain spotted fever in the United States during the years 1950–2005 is shown in Figure 1. For many years, approximately 500 cases occurred annually in the United States; then, after the introduction of specific therapy in 1948, the number of infected cases decreased to a low of about 200 in 1959, 1960, and 1961. This decrease, however, was primarily an artifact of the widespread use of broad-spectrum antibiotics early in the illness, which led to under-reporting and to the use of pesticides.

Beginning in 1969, there was a gradual increase in annual incidence in the United States. There were approximately 900 cases reported in 1975 and 1,067 in 1979.[18,46] Transformation of farms into housing developments and recreation of children and adults in wooded areas probably account for the added exposure to infected ticks, particularly in the southeastern Atlantic states, where the largest number of cases occur. This cyclic pattern has continued as seen in Figure 1. The number of cases has dramatically increased over the last few years and peaked with 2,288 in 2006, more than ever reported before in a single year. A recent seroprevalence study of children living in the southeast and south central regions of the United States found that 12% of children had *R. rickettsia* antibody titers of at least 64 and 4.3% had titers of at least 256,[47] suggesting an even higher prevalence of infection. There is no known explanation for the periodic rise and fall in the number of cases, nor for the marked recent increases (see Figure 1). A similar increase has been recently documented in Brazil and probably other areas of South and Central America.[48,49] Some authorities believe that the recent upward trend reflects increased awareness of tick-transmitted infections, increased diagnostic testing, and detection of antibodies induced by rickettsiae of low pathogenicity.

Rocky Mountain spotted fever in the United States and Sao Paulo typhus in Brazil are the most significant and clinically severe types of the tick-borne group. In the Eastern Hemisphere and elsewhere, the tick-borne rickettsioses are milder and are known variably as fièvre boutonneuse, South African, North Asian, Indian, Siberian, Queensland tick typhus, and others. Mite-borne rickettsialpox with its rodent reservoir is meddlesome rather than important. A large number of other spotted fever group rickettsia have been recently implicated or associated as causes of human disease ranging from mild to fatal.[50]

5.2. Epidemic Behavior and Contagiousness

The epidemiology of Rocky Mountain spotted fever is associated with the biology of the ticks that transmit *R. rickettsii*. Man is entirely an accidental victim and is in no way responsible for the maintenance of the infection in nature, which is due largely to ticks and the animals on which they feed. A number of species of ticks are found infected with *R. rickettsii* in nature. Four species of ixodid ticks have been long recognized as natural carriers of *R. rickettsii*: the Rocky Mountain wood tick, *Dermacentor andersoni*; the American dog tick, *D. variabilis*; the Lone Star tick, *Amblyomma americanum* and the cayenne tick, *Amblyomma cajennense* (in South America); and the rabbit tick, *Haemaphysalis leporispalustris*.[51,52] In 2005 *Rhipicephalus sanguineus,* the brown dog tick was recognized as an important vector in the transmission of *R. rickettsii* to humans in the United States.[53] *D. andersoni* is the principal vector in the West US and *D. variabilis* in the East US. Infected adult female ticks transmit the agent transovarially to many of their offspring. Ticks that become infected, either through the egg or by feeding on an infected mammal at one of the stages during their developmental cycle, harbor the rickettsiae throughout their life span, which may be as long as several years. Usually, cases occur sporadically within an endemic area, although there appears to be an increasing cluster-

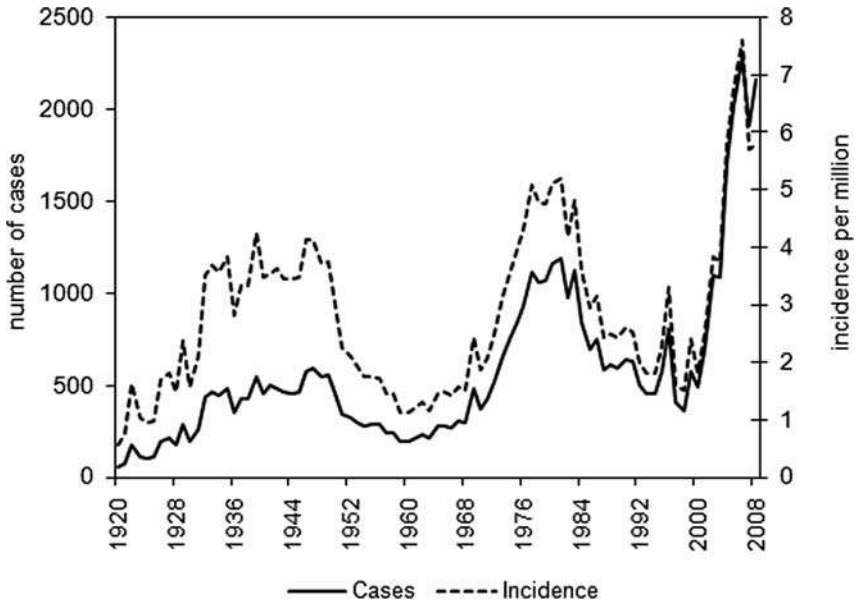


Figure 1. Rocky Mountain spotted fever cases reported in the United States, 1950–2008. Courtesy of the CDC.

ing of patients within families.[54,55] Person-to-person infection does not occur. Thus, the tick probably serves as a reservoir in addition to being a vector. Small animals such as wild mice and other rodents and domestic pets such as puppies may play an important role as reservoirs and in spreading rickettsiae in nature by infecting those new ticks that feed on them during the period of disease when rickettsemia is occurring, but the interval of rickettsemia is usually of limited duration. Most of the natural hosts show only inapparent infections with no overt clinical signs.[11]

5.3. Geographic Distribution

Spotted fever caused by *R. rickettsii* is limited to the Western Hemisphere, largely among wooded areas or fields that are good habitats for natural tick hosts. During the latter 19th century, the first reports were in Idaho and Montana, which led to the name Rocky Mountain spotted fever. The disease has been recognized throughout the United States except in Vermont, probably because of the lower prevalence of the tick vector in these regions. A change in tick vector can alter the distribution of Rocky Mountain spotted fever, as seen in Arizona in 2004 (see Figure 2).[53] It occurs in Canada, Mexico, Panama, Colombia, Argentina, and Brazil and is likely unrecognized in other Central and South American countries. Now, many more cases occur in the East, especially the southeastern and southcentral regions of the U.S., rather than in the West. In contrast, other species in the spotted fever group of *Rickettsia* account for spotted fever disease elsewhere in the world.

5.4. Temporal Distribution

There are seasonal variations in the incidence of cases of Rocky Mountain spotted fever. Most occur during the period of maximal tick activity, usually during the late spring and summer; however, confirmed cases are reported in every month of the year, particularly from the southeastern and south-central states and are particularly infrequent during the winter months in northern states.[11,46]

5.5. Age, Sex, and Occupational Factors

Differences in age and sex distribution of cases relate to exposure to ticks. In the western United States, a relatively higher proportion of adult males contract the disease because of outdoor occupational pursuits, whereas in the East, children and women are infected. This distribution is undoubtedly influenced by occupational propinquity to ticks and is not due to variations in susceptibility of various ages or sexes.[11,46,53]

The age and sex distributions are virtually the same for confirmed and unconfirmed cases. Nearly two-thirds of the cases are in persons less than 15 years of age likely a result of outdoor play exposures and 61% are in males. The case-fatality rates are significantly higher for non-whites (13.9%) than for whites (5.8%), higher for male patients (8.2%) than

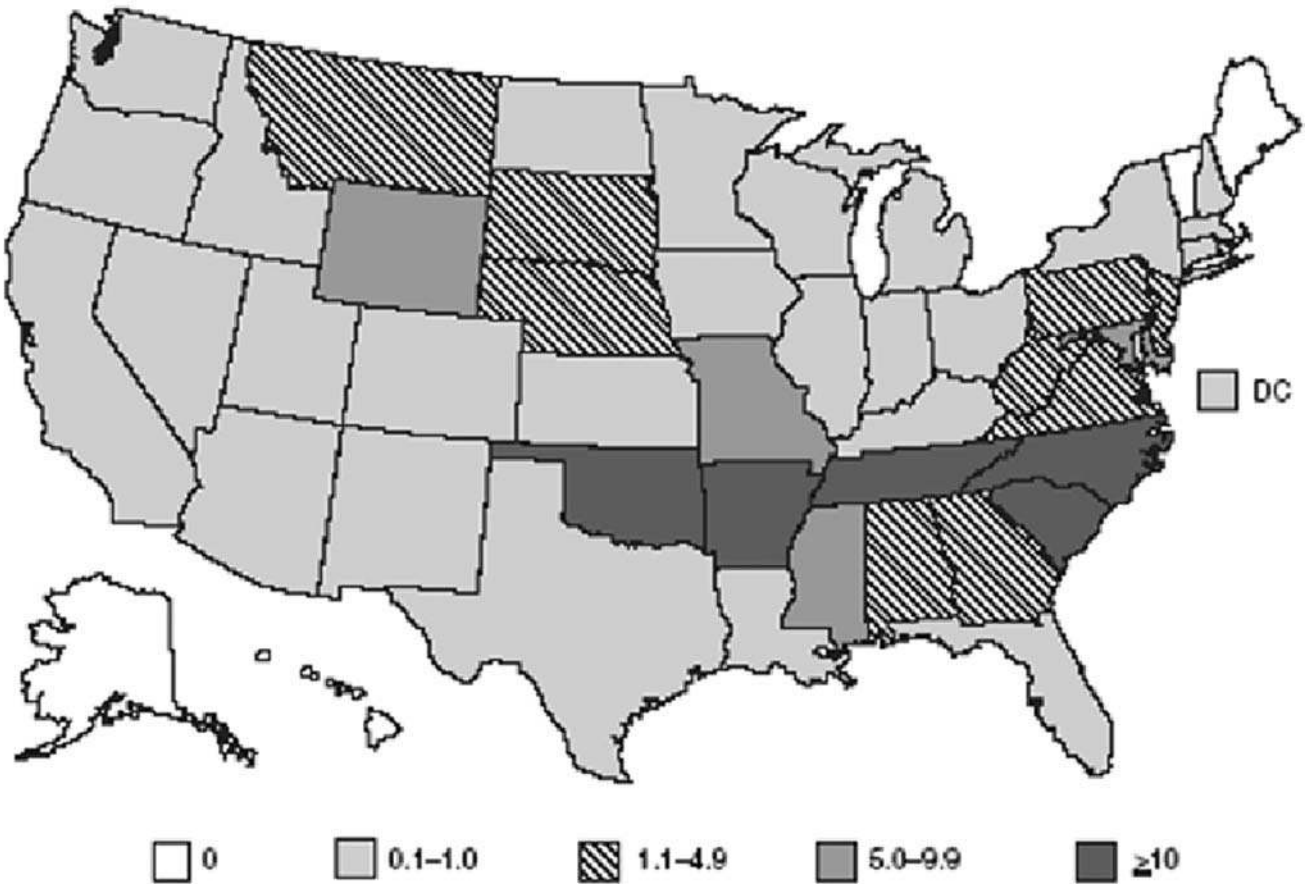


Figure 2. Geographic distribution and incidence (per 1,000,000 persons per year) of Rocky Mountain spotted fever in the United States for 1997–2002. Note the heavy localization in the southeastern and southcentral states.[20]

for female patients (4.5%), and higher for persons older than 30 years (13.9%) than for persons younger than 30 years (5.4%).[46] Delay in diagnosis with absence of an identifiable rash in the dark-skinned patient probably accounts for the variable mortality data.

6. Mechanisms and Routes of Transmission

Man is generally infected by the bite of an infected tick. The need for ticks to "reactivate" their infection from a nonvirulent immunizing phase in unfed adult ticks to a virulent infection-producing phase brought about by the ingestion of fresh blood probably explains why ticks do not cause illness unless they have attached and have fed for several hours. Infection may also be acquired through abrasions in the skin or through mucous membranes that become contaminated with infected tick feces or tissue juices. Hence, there is a hazard associated with crushing ticks between the fingers when removing them from persons or animals.[11] Quantitative studies conducted to test the efficacy of vaccines in volunteers showed that a very small number of viable rickettsiae given intradermally cause illness in man, i.e., 0.1 $GPIPID_{50}$ (50% guinea pig intraperitoneal infectious dose), which is about one rickettsial organism.[56]

7. Pathogenesis and Immunity

7.1. Pathogenesis

Rocky Mountain spotted fever in man follows infection through the skin via tick bite or rarely by respiratory tract with large aerosols after laboratory exposures. The bite of a tick usually transmits *R. rickettsii* after the tick has been attached for several hours. Infection may be acquired through an abrasion in the skin contaminated with infected tick feces or juices. Local lesions (eschars) develop with considerable rapidity at sites of arthropod attachment and initial multiplication of rickettsiae in scrub typhus, the

tick-borne rickettsioses of the Eastern Hemisphere, and rickettsialpox. A primary lesion occurs infrequently at the site of tick attachment in Rocky Mountain spotted fever, although recent observations suggest that a subset of these patients may be infected with another spotted fever group rickettsia such as *R. parkeri*.[26] An eschar also occurs as an initial lesion in tick-borne tularemia, which may confuse the diagnostic picture.[11,57]

After infection, illness occurs within a period varying from 3 to 12 days, the mean being about 7 days. A short incubation period usually indicates a more serious infection. Studies in volunteers have shown that patients with scrub typhus and Q fever have rickettsemia late in the incubation period prior to onset of fever.[58,59]

Similar events probably occur in all rickettsial diseases; circulating rickettsiae can be detected during the early febrile period in practically all patients. Little is known about the pathogenesis of infection during the midportion of the incubation period. Presumably, during this time in patients with Rocky Mountain spotted fever, a transient low-grade rickettsemia results from release of organisms multiplying at the initial site of infection, which then seeds infection within endothelial cells of the vascular tree. The presence of rickettsial antigens and tissue damage elicits an inflammatory response rich in lymphocytes and macrophages, both important components of ensuing cell-mediated immunity. The accumulation of these inflammatory cells at the site of infection leads to the characteristic histopathologic finding of vasculitis. Widespread vascular lesions that develop account for the physical findings such as rash and explain the pathophysiology of the infection as intravascular volume, plasma proteins, and electrolytes are lost into the interstitial spaces.

The exact mechanism by which rickettsiae enter and damage the endothelial cell is not known. *R. rickettsii* binds to an unknown cellular ligand, perhaps surface-expressed Ku70[42] and OmpA or OmpB play critical roles in adhesion.[38,39,42] This binding triggers or is followed by a mechanism that leads to endothelial cell invasion. Initially this process is mediated by phagocytosis associated with changes in intracellular calcium and the presence of phospholipase activity,[60,61] presumably of rickettsial origin since a phospholipase D-like gene encoding phospholipase activity is present in its genome.[62] Similarly, *R. prowazekii* contains a putative hemolyin, encoded in *tlyC*, that together with rickettsial Pld damages host cell membranes and may serve roles in (1) induced phagocytosis as a host-mediated reparative process and (2) escape from the phagosome into the cytoplasmic compartment before phagosome–lysosome fusion occurs.[62,63] Once within the endothelial cell, the rickettsiae mediate F-actin polymerization and become actively mobile.[43–45] The rickettsiae multiply by binary fission, and via actin polymerization the rickettsiae escape the cell, often prior to any evident cell injury. In most cases, endothelial cell damage is irreversible, and ultrastructural and biochemical evidence point to free radical and oxidative membrane injury.[64–66]

Later in the course of illness, the cascade of immune events is activated and matures. The macrophages attracted to the focus of infection undoubtedly process and present rickettsial antigens to T lymphocytes and secrete important cytokine mediators of inflammation including tumor necrosis factor-α and interferon-γ.[67] Data from animal models and in vitro studies suggest that cytokines such as tumor necrosis factor-α and interferon-γ act on infected cells to result in cell destruction and death of the rickettsiae contained within.[68] Cytokine-mediated alterations or damage coupled with rickettsia-mediated injury to critical endothelial cells compromises not only vascular flow but also enhances procoagulant activities, clinically resulting in thromboses or abnormalities in coagulation.[69] The activation of T lymphocytes, especially natural killer cells, leads to local secretion of interferon-γ, perhaps the most important cytokine factor elaborated by immune cells for enhancing intracellular rickettsial killing and protective immunity.[70,71] The balance of the degree of host injury (mediated by both the rickettsiae and host cytokines) and the degree to which rickettsiae are eliminated (via specific immune and nonimmune mechanisms) determines the overall amount of endothelial cell and vascular injury and is likely to be directly related to the clinicopathological findings and course in any given patient. This cellular and molecular pathogenetic sequence is consistent with in vitro experiments, animal models, and the experiences gleaned from human disease. In patients, infection is associated with induction of cutaneous delayed-type hypersensitivity reactions,[72] lymphocyte transformation,[73] and the development of serum antibody. However, antibody clearly lacks a protective effect and is unable to reverse an established infection although its contribution to protection against reinfection is now being re-evaluated.[30] The use of corticosteroids has some beneficial effect in human disease, probably by diminishing local inflammation and suppressing additional cytokine-mediated effects. When used in animal models, corticosteroids do not prevent death. Overall, it appears that induction of inflammation and immunity is beneficial to the infected human host, since the major goal is the elimination of the spread of intracellular infection by sacrificing the large numbers of infected endothelial cells and perhaps some critical vascular beds. When severe vasculitis is present, later in the illness, there is increased capillary permeability and vascular leakage that expands the extravascular space. This can lead to leakage of fluid into the lungs, brain, and peripheral tissues, particularly when excess fluids are administered intravenously.

7.2. Immunity

Second attacks of Rocky Mountain spotted fever have not been reported, although relapse infection after several days to weeks has developed in patients treated with antibiotics very early in illness. These are really continuing infections and not true recurrences.[56] A recurrent type of Rocky Mountain spotted fever such as Brill–Zinnser epidemic louse-borne type has not been reported. Clinicians should be alert to this possibility. Studies of cross-immunity in susceptible animals show protection among the various rickettsiae of the tick-borne group and rickettsialpox. Always, the protection derived from homologous antigens is more significant.

The underlying protective effectors after recovery from natural infection are not precisely identified; however, it is clear from in vitro and in in vivo experiments that natural killer cells and their secretion of interferon-γ play a critical role.[71] Infection and illness has been elicited in patients with high levels of preexisting antibody and preexisting in vitro correlates (lymphocyte transformation) of cell-mediated immunity.[73] The specific host and rickettsial factors that influence the development of an acquired protective immune state are actively being sought.

8. Patterns of Host Response

8.1. Common Clinical Features

A history of tick bite is elicited in approximately 58–80% of patients.[11,46,57] The onset of illness is usually abrupt with severe headache, a shaking chill, prostration, generalized myalgia, especially in the back and leg muscles,[11] nausea with occasional vomiting, and fever that reaches 102–104°F within the first several days. In some, the infection can be mild, accompanied by lethargy, anorexia, headache, and low-grade fever. These symptoms are similar to those of many infectious diseases and make specific diagnosis difficult during the first several days. Since *R. rickettsii* has only been isolated from humans with significant diseases, it is doubtful that many human inapparent infections occur, although seroprevalence data suggest otherwise.[47] However, the recognition of infections by *R. parkeri* that are mild and result in a seroconversion to *R. rickettsii* provides a potential explanation for high seroprevalence.[26]

8.1.1. Fever. In severe cases, fever continues for approximately 15–20 days; the febrile course in children may be shorter. Usually, the fever maintains a high continuous course with morning remissions that do not reach normal. Hyperpyrexia of 105°F or greater is usually an unfavorable sign, although death may occur when the patient is hypopyrexic with associated vasomotor collapse. Defervescence usually occurs over several days. Recurrent fever is unusual except in the presence of pyogenic complications.

8.1.2. Headache. Headache is usually general and severe, with frontal intensity. It persists during the 1st and 2nd weeks of illness in untreated cases; occasionally, it is mild. Malaise and muscle pain continue during the 1st week. Irritability is common, and the patient is often distracted during questioning and examination.

8.1.3. Rash. One of the most characteristic and helpful diagnostic signs is the rash, which usually appears on the 4th febrile day; the range is 2–6 days. Initial lesions are on the wrists, ankles, soles, palms, and forearms. They are macular, pink, and irregularly defined, and measure 2–6 mm (Figure 3). A warm compress on the extremities may accentuate a faint rash, and the rash is more prominent with elevated temperature. After about 12–24 h, the rash extends centrally to the legs, buttocks, arms, axillae, trunk, neck, and face. In epidemic typhus fever, the rash spreads from the trunk to the extremities. In about 2–3 days, the rash becomes maculopapular and assumes a deeper red hue (Figure 3). After 4 days or so, the rash is petechial and fails to fade on pressure (Figure 3). Often, the hemorrhagic lesions coalesce to form large ecchymoses (Figure 3). These lesions often form over bony prominences and may slough to form indolent, slow-healing ulcers. In convalescence, the petechiae fade to form hyperpigmented discolorations for several weeks. In mild cases, the rash does not become petechial or purpuric and may disappear within several days. Antibiotic treatment may abort the early exanthem; the late fixed lesions fade slowly with specific therapy. In some patients, particularly those with dark skin, a rash does not occur or is unnoticed. When tourniquets are applied to the extremities for several minutes or when the blood pressure is taken, additional petechiae may appear (Rumpel–Leade phenomenon); this is further evidence of capillary abnormalities. Photophobia and redness of the peripheral conjunctivae are often present.

8.1.4. Cardiovascular and Respiratory Signs. In severe cases, the pulse is rapid and the blood pressure is low. Circulatory failure with shock is an ominous sign and is an indication of the peripheral vascular atonicity and collapse. Ecchymotic skin lesions, cyanosis, and gangrene of the fingers, toes, genitalia, and buttocks are all unfavorable signs. The electrocardiogram shows changes indicative of myocardial involvement, and arrhythmias of various types indicate myocarditis, which may lead to cardiac arrest and death. In severely ill patients, edema of the arms, legs, and face indicates vascular decompensation and capillary abnormalities.



Figure 3. Evolution of skin rash in RMSF. Most physicians are educated to believe that the predominant rash of RMSF is petechial; however, a spectrum of findings is more typical, ranging from early macular or maculopapular (*top left*) rashes to petechial (*top right* and *middle left*) or purpuric lesions with advanced stages or fatal RMSF (*bottom*, courtesy Grover Hutchins, M.D.). *Rickettsia rickettsii* can be demonstrated in endothelium of dermal vasculitic lesions by immunohistochemistry (*middle right*). From Dumler and Walker, *N Engl J Med*, 2005; 353: 587–594 (Copyright © 2005 Massachusetts Medical Society. All rights reserved. Bottom panel with permission from G. Hutchins, M.D.).

Respiration is usually rapid, and a nonproductive cough with pulmonary rales is indicative of a form of rickettsial pneumonitis. Lung consolidation is rare. Judicious use of intravenous fluids is warrantred as they may provoke pulmonary edema.

8.1.5. Hepatic and Renal Signs. Hepatomegaly occurs; usually, there is no jaundice. Hypoalbuminemia results from leakage of albumin into subcutaneous tissues and abnormal hepatic function. Oliguria, anuria, and azotemia are indicators of vascular decompensation and renal involvement. Acute renal failure is highly associated with mortality.[17]

8.1.6. Gastrointestinal Signs. Abdominal distension is frequent and occasionally some degree of intestinal ileus is observed. Constipation is usual. More acute manifestations with abdominal pain may suggest the presence of either appendicitis or cholecystitis.

8.1.7. Neurological Signs. Headache, restlessness, insomnia, and back stiffness are common neurological manifestations. Coma, muscular rigidity, athetoid movements, convulsive seizures, and hemiplegia (rare) are grave signs and indicate the presence of encephalitis. Deafness during the acute illness and in convalescence is common. Most of the CNS signs abate with time.

8.2. Clinical Course and Complications

In mild and moderately severe cases, the illness abates in about 2 weeks. Convalescence is rapid. If death occurs, it is usually during the latter part of the 2nd week as a result of toxemia, vasomotor weakness, and shock or renal failure with azotemia. In a few patients, the course is rapidly fulminant with death outdistancing treatment, occurring as early as the 6th day of illness.[11,46]

The most significant complication comes from delayed diagnosis of RMSF.[17,20] Even in areas with a higher than average prevalence, where physicians' awareness of the illness is increased, the diagnosis of RMSF is often delayed and the standard therapy for pediatric patients is not always given.[74] Delayed diagnosis and treatment affects outcome and increases mobidity and mortality.

8.3. Diagnosis

During the early stages of infection before the rash has appeared, differentiation from other acute infections is difficult. History of tick bite while living or traveling in a highly endemic area is helpful.

Cutaneous manifestations can be used to help distinguish Rocky Mountain spotted fever from other febrile exanthems. The rash of meningococcemia resembles that of Rocky Mountain spotted fever in certain aspects, since it is macular, maculopapular, or petechial in the chronic form, and petechial, confluent, or ecchymotic in the fulminant

type. The meningococcal skin lesion is tender and develops with extreme rapidity in the fulminant form, whereas the rickettsial rash occurs on about the 4th day of disease and gradually becomes petechial. High blood leukocyte counts are common in meningococcal infections. The exanthem of rubella rapidly becomes confluent, while that of rubeola almost never becomes petechial. The exanthem of varicella or variola is first erythematous and later becomes vesicular. The rose spots of typhoid fever are usually on the lower chest or abdomen and remain delicate, without hemorrhagic character. Rocky Mountain spotted fever skin lesions, in contrast to those of typhoid, begin on the periphery of the skin and later become petechial. The rash of infectious mononucleosis is usually morbilliform on the trunk and rarely becomes petechial. Lymphadenopathy and atypical lymphocytes in the blood are differentiating features. Murine typhus and cat flea typhus (caused by *Rickettsia felis*) are milder than Rocky Mountain spotted fever; the rash is less extensive and is usually nonpurpuric and nonconfluent; renal and vascular complications are uncommon. Not infrequently, differentiation of these two rickettsial infections must await the results of specific serological tests. Epidemic typhus fever is capable of causing all the pronounced clinical, physiological, and anatomical alterations seen in cases of Rocky Mountain spotted fever, i.e., hypotension, peripheral vascular failure, cyanosis and gangrene of digits, renal failure with azotemia, and neurological manifestations. However, the rash of classic typhus is noted initially in the axillary folds and on the trunk and later extends peripherally, rarely involving the palms, soles, or face. The serological patterns in these two diseases are distinctive when specific rickettsial antigens are employed in tests. Moreover, louse-borne typhus is not recognized in the United States except in the form of Brill–Zinsser disease (recurrent typhus fever) and the rare cases associated with flying squirrels (sylvatic typhus). Rickettsialpox, and now *R. parkeri* infection, although both caused by members of the spotted fever group, are usually readily differentiated from Rocky Mountain spotted fever by the initial lesion, the relative mildness of the illness, and with rickettsialpox, the early vesiculation of the maculopapular rash.

The laboratory findings in Rocky Mountain spotted fever are nonspecific. However, several features may aid in establishing the differential diagnosis. Rocky Mountain spotted fever is often associated with normal or low leukocyte counts and significant left-shifts in patients who are quite obviously ill and would be expected to have leukocytosis. Platelet counts are frequently diminished. Careful evaluation of coagulation tests may reveal mild abnormalities, and despite the occasional clinical appearance of a state suggestive of disseminated intravascular coagulation, fibrinogen levels in serum are usually within normal ranges. Electrolyte abnormalities occur often, particularly hyponatremia, and these are best explained on the basis of appropriate antidiuretic hormone release in response to the loss of intravascular volume into interstitial spaces and by the actual redistribution of electrolytes into third space tissue compartments. Elevations in serum hepatic transaminases, creatine phosphokinase, lactate dehydrogenase, and other enzymes are likely to result from bystander inflammatory injury to hepatocytes or injury secondary to ischemia and hypoperfusion. The development of hyperbilirubinemia and hepatic failure is an ominous sign indicative of severe, probably impending fatal infection. Abnormalities in urea nitrogen and creatinine levels usually indicate renal injury secondary to hypovolemia; however, rickettsial interstitial nephritis is well recognized and may be a contributor in some circumstances. Careful intrepretation of laboratory findings and correlation with the clinical examination are imperative. Most often, the clinical findings and laboratory examination will reveal evidence of a systemic disease, findings that will discourage unwarranted surgical interventions.

Scrub typhus, African tick-bite fever, and boutonneuse fever (Mediterranean spotted fever) patients are being increasingly reported in the United States among travelers from the Far East[75] and Africa.[76,77] The clinical features may simulate Rocky Mountain spotted fever when severe, especially for scrub typhus and boutonneuse fever; however, when detected early, antibiotic treatment is very effective. The vector of scrub typhus is a mite and ticks are the vectors of African tick-bite and boutonneuse fevers.

9. Control and Prevention

9.1. Vector Control

Avoiding or reducing the chance of contact in tick-infested areas of known endemicity is the principal preventive measure. When this is impractical, area control of ticks may include the following prophylactic measures:

1. Spraying the ground with acaricides for tick control. Although there are environmental objections to the use of residual insecticides, such procedures may be warranted under special conditions.
2. Application of repellents such as N, N-diethyl-m-toluamide (DEET) or dimethyl phthalate to clothing and exposed parts of the body, or in very heavily infected areas, the wearing of clothing that interferes with the attachment of ticks, i.e., boots and a one-piece outer garment preferably impregnated with repellent.[11]
3. Daily inspection of the entire body, particularly of children, including the hairy parts, to detect and remove attached ticks. Care should be taken in removing attached ticks to avoid crushing the arthropod, which will contaminate the bite wound. Gentle traction

with small forceps applied close to the mouth parts allows ready extraction.[78] The skin area should be disinfected with soap and water or other antiseptics such as alcohol. These precautions should be employed in removing engorged ticks from dogs and other animals because infection through minor abrasions in the hands is possible.

9.2. Chemotherapy and Prophylaxis

The question is often raised whether antibiotics should be given prophylactically after human exposure to virulent *R. rickettsii*. In guinea pigs, a single dose of oxytetracycline prevented the disease when the antibiotic was given shortly before expected onset. However, relapses occurred when treatment preceded expected onset by 48 h or more.[79] This regimen is not recommended. After a tick bite in a known endemic area, an exposed person should be observed for signs of fever, headache, prostration, and rash; therapy is very effective at the early stage of illness.

Tetracycline (doxycycline) is specifically effective for treatment of Rocky Mountain spotted fever. It is rickettsiostatic, not rickettsicidal. Clinical manifestations subside promptly, if initiated early when the rash first appears. Doxcycline is preferred over tetracycline since it is associated with lower tooth discoloration and less frequent dosing, significantly reducing concern over treatment in children less than 8 years. Historically, chloramphenicol has also been considered effective, although this is now a controversial point.[80,81] While some in vitro studies have suggested the possibility that fluoroquinolones and macrolides could be useful treatments for RMSF, no clinical trials to determine efficacy in vivo have been conducted. Any seriously ill child or adult who lives in a wooded area with unexplained fever, headache, and prostration, with or without a history of tick contact should be suspected of having Rocky Mountain spotted fever. Specific treatment should be initiated after blood is taken for diagnostic tests. Optimal antibiotic regimens include twice daily doxycycline 100 mg (adult dose) or 2.2 mg/kg (child dose) orally or by intravenous injection. Therapy is continued until the patient has improved and has been afebrile for about 48 h. Intravenous preparations are used in patients too ill to take oral medications.

Although not supported by any clinical trials, critically ill patients first observed late in the course of illness may be given large doses of corticosteroids (for treatment of the vasculitis) in combination with specific antibiotics for about 3 days. Such patients often show circulatory collapse, oliguria, anuria, azotemia, hyponatremia, hypochloremia, edema, and coma. In such patients who manifest increased capillary permeability, intravenous fluids should be given cautiously to avoid increasing pulmonary and cerebral edema. In mildly or moderately ill patients, these pathophysiological abnormalities are not present.

9.3. Immunization

Spencer and Parker[8] were aware that infected ticks, when they first come out of hibernation, produce either no or low-grade infections in guinea pigs. They found that one infected egg from an infected female tick when triturated and inoculated into a guinea pig produced spotted fever and that suspensions of infected ticks treated with phenol protected guinea pigs against virulent challenge. Spencer and Parker[8,82,83] tested the new vaccine on themselves and showed that a sample of their blood, taken after vaccination, mixed with virulent rickettsiae protected guinea pigs against infection. They developed a tick-derived vaccine, inactivated and preserved with 1.6% phenol and 0.4% formalin. The vaccine was difficult to produce and expensive, originally costing $20.00 per injection. Studies of efficacy were carried out over a number of years. Although no carefully controlled experimental trials were conducted, it appeared, on the basis of differences of attack rates in small groups of vaccinees and control volunteers, that the vaccine prevented the mild type of spotted fever and sharply reduced the mortality in the highly fatal type.[84] The vaccine was moderately reactive, producing malaise, nausea, slight fever, arthralgia, myalgia, and serum sickness in about 1% of recipients.

Cox developed an inactivated vaccine prepared from rickettsiae grown in yolk sacs of fertile hens' eggs. This was the standard preparation for a number of years.[85] Immunization consisted of a course of three injections of 1.0 ml each given subcutaneously at weekly intervals. Vaccination was preferably performed in the spring before the advent of the spotted fever season, and a 1.0-ml stimulating dose was recommended each year for those at risk. There was little reaction from the vaccine. This chick-tissue vaccine produced commercially (Lederle) was not conclusively tested in the field, and furthermore, the relationship between the immunity demonstrated in guinea pigs and that induced in man following administration of vaccine was inconclusive.

A quantitative trial of inactivated vaccines made from infected tissue and embryonated hens' eggs was conducted in volunteers followed by a challenge with virulent (viable) *R. rickettsii*.[56] Humoral antibodies appeared in a low percentage of vaccinees after use of either available vaccine. It was noted in the vaccinated group that when they were injected with a few virulent rickettsiae 3–6 months after a full course of vaccination, the incubation period was prolonged and the frequency of early clinical relapse was lessened.

Vaccinated volunteers were not protected (when compared with controls) against development of clinical illness. In vaccinees, the clinical illness appeared to be shortened,[56] which coincided with the earlier findings of Parker.

There was great hope that a cell-culture-derived, inactivated *R. rickettsii* vaccine would provide greater protective effect than the previously approved vaccines. Initial studies in nonhuman primates suggested efficacy; however, when administered to humans as a challenge trial, the results were disappointing.[72] No vaccinated volunteer was protected from illness, despite induction of serum antibody and in vitro correlates of cell-mediated immunity.[73] The interpretation of all the studies suggests that the vaccine might be useful if the regimen were modified to include more rickettsial mass and more booster immunizations.

To date no proven or approved vaccine for Rocky Mountain spotted fever exists. Research designed to investigate the important rickettsial factors toward which protective immune responses may be directed is in progress. The successful elucidation of Omps and perhaps Pld as targets for human vaccine development using recombinant proteins has revealed promising results in animal models.[41,62,86] A clearer understanding of the mechanisms by which intracellular infections are resolved and protective immunity is established is mandatory before another human vaccine can be proposed. Until then, the need will not diminish, but other methods for control will be required.

10. Unresolved Problems

Specific chemotherapy of the rickettsial diseases was achieved in 1948. This event inadvertently diverted attention from pursuit of studies aimed at elucidating pathogenesis, pathophysiological abnormalities, and control by vaccines. Some unanswered puzzles awaiting solution are

1. What is the nature of the increased capillary permeability and the cause of the characteristic vascular lesion? The specific molecular events leading to *Rickettsia*-mediated host membrane damage and the relative contributions of host-derived "pathological" cytokine responses and their reversal need clarification. There are favorable advances in understanding control of illness mediated by the untoward effects of cytokines. Are the changes a result of direct effects by rickettsiae within endothelial cells, an action of their manipulation of cellular signaling processes, a combination of both, or an immunopathologic reaction? Evidence does not permit the exclusion of any one mechanism.
2. Hematologic findings in severely ill patients with spotted fever and typhus show a disseminated intravascular coagulation defect late in illness. Its mechanism and the relationships of complement abnormalities merit study.
3. Therapy for early cases is simple and effective. For seriously ill patients during later stages, there is a need for better antitoxemic measures, which will evolve only when the nature of the pathological lesions and the toxic manifestations are understood. Short-term high-dose steroid treatment for several days combined with antibiotics is helpful and is unassociated with sequelae.
4. Spotted fever and typhus group rickettsiae persist within human cells, presumably within macrophages; Brill–Zinsser disease is a recurrence of epidemic typhus years after the initial infection. This is a remarkable example of microbial persistence. Conceivably, there is a Brill–Zinsser pattern for other rickettsial diseases such as spotted fever, murine and scrub typhus, and Q fever. An understanding of the intracellular growth requirements might provide leads to the complete inactivation of rickettsiae. An important lead will be the cultivation of rickettsiae on artificial media.
5. There is a need for better biological vaccines for prevention of typhus, spotted fever, and Q fever. Inactivated Q fever vaccine is effective but causes reaction on repeated inoculation. A tissue-culture-derived inactivated vaccine for spotted fever is under development and appears promising. This system may be useful for production of better epidemic typhus vaccines. Live strain E vaccine for typhus is effective but reactive, and reversion to virulence has been reported.[87] There are no effective vaccines for scrub typhus. Whether the threat of some rickettsial diseases for humans warrants research directed toward developing attenuated viable vaccines should be carefully considered.
6. The interrelationships of rickettsiae in animal hosts, vectors, and other environmental factors for epidemic and murine typhus, Rocky Mountain spotted fever, and Q fever are really under-developed. For example, there may be a significant reservoir of *R. prowazekii* other than man, such as flying squirrels. Fleas or lice may serve as vectors.
7. Better systems are needed for genetic modification of rickettsia to either inactivate rickettsial genes or transfer mutant genes into rickettsiae. This would allow a modern molecular equivalent of Koch's postulates to be applied for the understanding of rickettsial molecular and cellular pathogenesis.

Current work by several investigators is directed to these questions.

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CHAPTER 32

Salmonellosis: Nontyphoidal

Michael F. Lynch and Robert V. Tauxe

1. Introduction

Nontyphoidal salmonellosis traditionally refers to disease caused by any serotype of organisms in the genus *Salmonella*, other than *Salmonella* Typhi, the causative agent of typhoid fever (see Chapter 42). The most common manifestation of nontyphoidal salmonellosis is acute enterocolitis, but the organism can cause focal infection, bacteremia, meningitis, as well as "enteric fever" that may be clinically indistinguishable from that caused by *S.* Typhi. Nontyphoidal salmonellosis is a disease of considerable clinical and public health importance. An estimated 1.4 million cases of salmonellosis occur each year in the United States, of which a small fraction are cultured and reported. The direct patient care and lost productivity costs alone have been estimated to exceed $2 billion annually, but when one considers the added costs of plant closings, product recalls, and losses of food production, the true economic impact of salmonellosis is likely to be substantially greater.[1]

Over 2,000 serotypes of *Salmonella* have been described.[2] The nomenclature of *Salmonella* has undergone considerable evolution over recent years, which has led to some confusion. By DNA hybridization, two species, *Salmonella enterica* and *Salmonella bongori*, have been identified.[2] Almost all clinical *Salmonella* isolates belong to *S. enterica,* which is further subdivided into six subspecies. Most *Salmonella* serotypes associated with human diseases belong to subspecies 1 (subspecies *enterica*), but strains in subspecies 3, once referred to as the Arizona group, are occasionally isolated. Previously, the genus *Salmonella* was classified into three primary species which were called *Salmonella typhi*, *Salmonella cholerae-suis*, and *Salmonella enteritidis*; Arizona organisms were in a separate genus. The species *S. typhi* and *S. cholerae-suis* consisted of a single serotype each; the remaining 2,000 serotypes were in the species *S. enteritidis*. The formal designation of a serotype is *S. enterica* subspecies *enterica* serotype Typhimurium or *S. enterica* subspecies *enterica* serotype Enteritidis.

To avoid the awkwardness present in this and previous nomenclatures, we will designate each serotype directly, without reference to the full species name. Thus, *S. enterica* subspecies *enterica* serotype Typhimurium is written *S.* Typhimurium and *S. enterica* subspecies *enterica* serotype Enteritidis is written *S.* Enteritidis. Serotypes outside of subspecies 1 are now referred to by their antigenic formulae. The two different organisms formerly known as *Salmonella* Paratyphi B and *Salmonella* Java actually share the same serotype, but are distinguished by the tartrate test; they are now referred to explicitly as tartrate-positive or -negative variants of *S.* Paratyphi B.

Salmonellae probably evolved with the reptiles and birds and are well adapted to a variety of host animals. The diversity of serotypes reflects this diversity of hosts, as many serotypes have a narrow host range. For example, clinically important infections due to *S.* Typhi occur only in humans, and humans are the only known reservoir for this organism. The primary reservoir of *Salmonella* Gallinarum and *Salmonella* Heidelberg is chickens; the reservoir of *Salmonella* Choleraesuis is pigs; for *Salmonella* Bovismorbificans it is cows; IV 50:g,z51: (*Salmonella* Marina) is the marine iguana; and Paratyphi B var. L(+) tartrate+ (*S*. Java), *S*. Urbana, and *S*. Litchfield are predominantly turtle associated. Nonetheless, all serotypes should be considered potentially pathogenic for humans and animals. In addition to distinctive host ranges, many serotypes exhibit unique patterns of virulence, antibiotic resistance,

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and geographic distribution that make the epidemiology of *Salmonella* particularly fascinating and complex.[3]

2. Historical Background

Salmon and Smith[4] reported the isolation of "hog cholera bacillus," later named *S.* Choleraesuis, from diarrheic swine in 1885. Three years later, Gaertner[5] reported the first foodborne outbreak of nontyphoidal salmonellosis, in which 58 persons fell ill after eating raw meat from an ill cow; "Gaertner's bacillus," later named *S.* Enteritidis, was isolated from the meat, and from the spleen of a man who died in the outbreak. In 1889, Loeffler isolated *S.* Typhimurium during an outbreak of diarrheal illness in a laboratory mouse colony; the same organism was demonstrated among victims of a meat-associated outbreak in Aertrycke, Belgium, in 1899.[6,7] By the turn of the century, the pathogenic potential of *Salmonella* for humans was widely appreciated in Europe, and following World War I, salmonellosis emerged as a common public health problem on that continent. Between 1923 and 1938, 374 outbreaks due to 11 different serotypes of nontyphoidal salmonellae were reported in the United Kingdom.[6] *Salmonella* Enteritidis infections associated with duck eggs became common in Europe and led to the decline in popularity of this food before World War II.[8] In 1941, Kauffmann[6] presented an expanded version of White's approach to the great antigenic diversity of *Salmonella*. The Kauffmann–White schema defined the antigenic formula of each serotype, and provided the widely used nomenclature that we have followed here.

In the United States, nontyphoidal salmonellosis did not emerge as a public health problem until after World War II. The number of reported clinical cases increased from 502 in 1942, the first year that the condition was made nationally reportable, to 65,347 in 1985 (the year of a large milk-associated outbreak) and declined to 45,322 in 2005 (Figure 1). Over the same time, typhoid fever, caused by *S.* Typhi, became rare. In 1962, following a large multistate outbreak of egg-associated *Salmonella* Derby infections, a second national surveillance system for *Salmonella* was instituted, based on reports of laboratory isolates, which include serotype. This surveillance system, coupled with intensive epidemiological investigations, has helped to clarify our understanding of the complex routes of *Salmonella* transmission. As a result of this improved understanding, specific control measures have been implemented. The rapid rise in reported incidence of salmonellosis in the 1970s and 1980s, slight decline in the 1990s and stable rates in the first few years of the 21st century, indicates the need for further improvements in our efforts to control this pathogen of the modern era.

3. Methodology

3.1. Sources of Mortality Data

Data are limited on the mortality from salmonellosis in the United States. Deaths due to salmonellosis are not routinely reported to the Centers for Disease Control and Prevention (CDC) national surveillance systems. Mortality can be estimated from periodic nationwide surveys of sentinel counties and established active surveillance in sentinel sites (see Section 3.3), from death certificates, and from outbreak investigations. The surveys of sentinel counties and active surveillance in sentinel sites probably give the most reliable overall estimate of mortality. The sentinel county studies yielded a mortality rate of between 0.5 and 1.4%, which is similar to that found in 16 years of surveillance in Massachusetts,[9] but lower than the 4.1% mortality rate observed in New York between 1939 and 1955.[10] More recently, a mortality rate of 0.6% was estimated from active surveillance in five sentinel sites for the years 1996–1999, accounting for an estimated 400 deaths due to salmonellosis annually in the United States.[11] In outbreaks, the typical case-fatality rate is 0.1% of affected persons.[12] This is lower than the mortality for reported cases because outbreak investigations identify many milder cases that would not be cultured, diagnosed, or reported had they occurred as sporadic cases. Approximately 20–40 deaths per year due to salmonellosis are identified from death certificate data.[13] Death certificates may not reflect the bacteriologic diagnosis in patients dying of gastroenteritis, dehydration, sepsis, or meningitis if appropriate cultures are not obtained or culture results are not available at the time of death. Deaths due to *Salmonella* may be hidden in those attributed to septicemia, meningitis, or cardiopulmonary arrest.

3.2. Sources of Morbidity Data

Since 1962, the laboratory-based National *Salmonella* Surveillance Program has been conducted by the CDC working jointly with the Council of State and Territorial Epidemiologists, the Association of State and Territorial Public Health Laboratory Directors, the US Department of Agriculture, and the US Food and Drug Administration. This program is based on weekly reports of *Salmonella* isolations from the states and from the District of Columbia, and

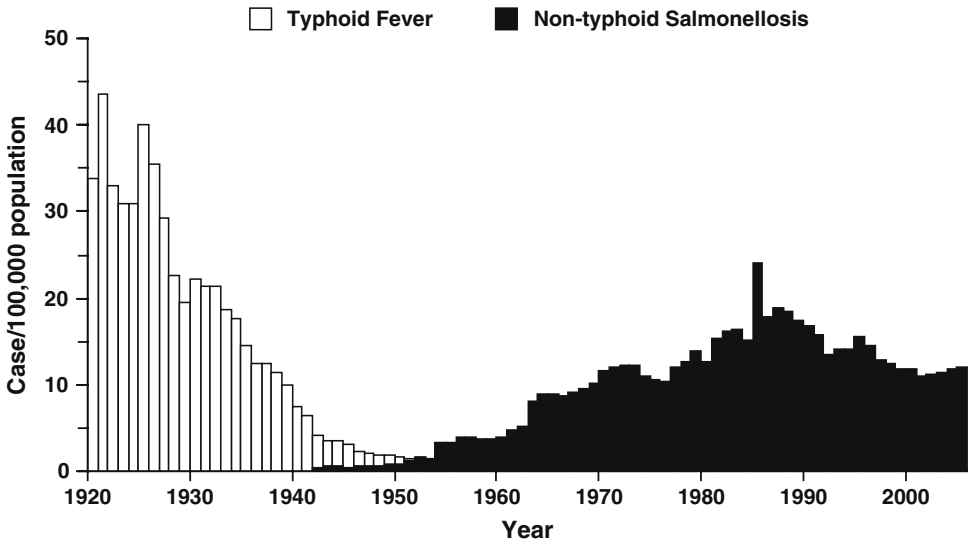


Figure 1. Reported incidence of clinical typhoid fever and nontyphoid salmonellosis in the United States from 1920 to 2005. Data from CDC [13] (1985 peak of nontyphoidal salmonellosis includes cases from massive outbreak with >16,000 culture-confirmed infections.[38]).

regular summaries of nonhuman isolates from the Food and Drug Administration and the US Department of Agriculture. All laboratories are requested to forward clinical isolates of *Salmonella* to the state or regional laboratory for serotyping, and the results are reported along with information on sex, age, county of residence, and site of isolation. Since 1993, these data are transmitted, collected and analyzed electronically via the Public Health Laboratory Information System (PHLIS).[14] The purpose of this laboratory-based surveillance program is to define patterns of endemic salmonellosis in the United States, to identify trends in disease transmission, and to monitor control efforts. The predecessor to this system, based on physicians' reports of cases, began in 1942 when nontyphoidal salmonellosis became a notifiable disease, and continues to function in parallel. The clinical case-reporting system yields comparable numbers of cases, but because case definitions vary from state to state and the reports do not include serotype, it is of limited epidemiological use.

Limitations and biases exist in the laboratory-based data as well. The reports do not discriminate between symptomatic and asymptomatic cases, and cases of salmonellosis without laboratory confirmation are not included. Several factors influence whether a case of salmonellosis will be reported to the *Salmonella* surveillance system, including the following: the severity of illness, access to medical care, and the association with a recognized outbreak. Physicians may be more likely to obtain stool cultures from infants and elderly persons with gastroenteritis than from young adults, and illness in these age groups may be oversampled. By comparing the number of cases detected in outbreak investigations with the number of outbreak-related cases that are reported to the *Salmonella* surveillance system, it is possible to estimate the sensitivity of the system. Reviewing a series of outbreaks in the 1960s, Aserkoff[15] estimated that 1% symptomatic infections were reported to the laboratory-based surveillance system. Using a variety of methods, including extrapolation from the carrier rate, estimating sequential error in the reporting system, and reviewing outbreaks, Chalker and Blaser[16] estimated that between 1 and 5% of infections are reported. From surveys of healthcare seeking behavior of persons with diarrhea, physician testing practices and laboratory isolation and reporting rates in active surveillance sites during 1996–1999, Voetsch[11] estimated that each reported case of laboratory-confirmed *Salmonella* infection represented approximately 38 unreported cases.

3.3. Surveys and Investigations

Because of the inherent limitations of nationwide surveillance, other studies have been performed and active surveillance has been established in selected sites to obtain a more detailed picture of the epidemiology of salmonellosis. In 1979–1980, the CDC performed a survey of all persons from whom *Salmonella* was isolated in a randomly selected, stratified sample of counties.[17] A detailed epidemiological questionnaire concerning exposures, symptoms, and complications, was administered to patients and the isolates were

examined for antimicrobial resistance. A similar survey was repeated in the same sentinel counties at 5-year intervals since 1984–1985,[18] 1989–1990,[19] and 1994–1995. Following this model, in 1996, the Foodborne Disease Active Surveillance Network (FoodNet) was established in five states.[20] Expanded to 10 states in 2005, FoodNet conducts active surveillance for *Salmonella* infections and other foodborne diseases. In addition, a proportion of *Salmonella* isolates causing these infections are examined for antimicrobial resistance through the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS).[21]

The most detailed information on the epidemiology of salmonellosis has come from outbreak investigations. These investigations provide the opportunity to determine vehicles of transmission, the spectrum of illness, and risk factors for infection and for poor clinical outcomes. Case–control methods and molecular biological techniques for precisely defining the strains involved have made the outbreak investigation an occasion for extraordinarily fruitful research,[22] and outbreak investigations have in turn spawned systematic studies. Examples include studies of the decline of turtle-associated serotypes[23] and studies of the dissemination of chloramphenicol-resistant *Salmonella* Newport following an outbreak in California.[24]

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification. Members of the genus *Salmonella* are gram-negative, flagellated, nonsporulating, aerobic bacilli. *Salmonella* can usually be directly isolated from primary plates inoculated with stool or rectal swabs from persons with *Salmonella* enterocolitis. The diagnostic yield may be slightly higher if one uses an aliquot of 5–10 g of feces rather than a rectal swab, particularly when attempting to identify *Salmonella* carriers, who may be shedding only small numbers of organisms. As carriers may shed *Salmonella* intermittently, culturing specimens on two or more occasions may increase the likelihood of detection. In clinical laboratories, an enrichment broth is also recommended to increase the isolation of *Salmonella* from stool specimens.[25] Three selective enrichment media are in widespread use to isolate *Salmonella*: tetrathionate broth, tetrathionate broth with brilliant green, and selenite F broth. Many microbiologists prefer to use either selenite or tetrathionate without the brilliant green dye because neither inhibits *S.* Typhi, in contrast to tetrathionate brilliant green broth. Numerous selective plating media, varying from low to high selectivity, have been used to isolate *Salmonella* from fecal specimens. The purpose of these selective media is to suppress the growth of other Enterobacteriaceae. MacConkey, eosin–methylene blue (EMB), and deoxycholate agar are media of low selectivity. *Salmonella–Shigella* (SS), Hektoen enteric (HE), and xylose–lysine–desoxycholate–citrate (XLD) agars are widely used to screen for *Salmonella* and *Shigella* because they support the growth of both organisms and are considered moderately selective media. When laboratory resources permit the use of an additional plate, highly selective media such as brilliant green or bismuth sulfite agar may be superior to the less selective media. Bismuth sulfite agar is the preferred medium for isolating *S.* Typhi. For specimens obtained from a normally sterile source such as blood or cerebrospinal fluid (CSF), using selective media has no advantage.

Following accepted microbiologic methods, colonies that are suspicious for *Salmonella* should be further identified by a chemical test. At least two representatives of each type of suspicious colony should be selected and transferred to either triple sugar iron (TSI) or Kliger's iron (KI agar). Lysine iron agar (LIA) indicates the decarboxylation or deamination of lysine and the production of hydrogen sulfide, and is particularly helpful when used in conjunction with TSI agar for the presumptive identification of *Salmonella*. The usual reaction of *Salmonella* on TSI or KI will be an alkaline slant and acid butt, and they will produce gas and H_2S. Lactose-positive strains may occasionally occur that will produce an acid slant and do not produce H_2S or gas. On LIA, the usual reaction is an alkaline slant and alkaline butt with production of H_2S. Isolates can be screened for *Salmonella* antigens using a polyvalent O or H antiserum. Colonies with typical TSI and LIA reactions that react to polyvalent O and H antisera are presumptively considered to be *Salmonella*.

If stool specimens must be transported, as is often necessary in epidemiological investigations, a swab coated with feces should be placed in a transport medium and held refrigerated until it can be examined. Commonly used transport media include Stewart's, Amies, Cary-Blair, and buffered glycerol saline. Of these, Cary-Blair is recommended as a general transport medium in the United States.[25]

Rapid diagnostic tests using enzyme immunoassay, latex agglutination, or other immunologic methods to detect *Salmonella* in feces and in foods are under development. When these methods are used in the clinical or public health setting, it will be critical to also isolate the organism itself, so that serotype, subtype, and antimicrobial resistance can be determined.

3.4.2. Serological Diagnostic Methods. Salmonellae possess somatic (O) and flagellar (H) antigens, as do other Enterobacteriaceae. The O antigenic determinants define the serogroup, and the H antigens further break these

down into more than 2,000 serotypes, according to the Kauffmann–White schema. This great variety is achieved in part because most *Salmonella* have a unique ability to express at least two separate sets of flagellar antigens. When they encounter antibodies to their usual flagella, they produce a second set, with unrelated antigens. Variations in each set of antigens and in the order in which they appear define the large number of serotypes present in each serogroup. Each serogroup has a major determinant and one or more minor somatic antigens. Serogroups A–I have been identified; most serotypes that cause human disease fall into serogroups A–E. Many clinical laboratories can readily identify serogroup using commercial polyvalent grouping antisera. Further identification of specific serotype is usually performed by a public health laboratory or other reference laboratory. Serotype determination is extremely useful in epidemiological investigations since many serotypes have specific reservoirs, host ranges, virulence, and age specificity. DNA-sequence-based approaches to defining serotypes of *Salmonella* are under active development and may soon begin replacing the traditional approach based on rabbit antisera.[26]

Since the 10 most common serotypes account for 66% of human isolates in the United States, laboratory techniques for further subtyping strains within a common serotype are often useful for epidemiological purposes.[22] Antimicrobial resistance patterns are the simplest method of further characterizing an epidemic strain. Plasmid profiles have proved useful in defining outbreak strains.[27–30] Phagetyping schemes for *S.* Typhimurium and other serotypes have been used extensively for epidemiological purposes in England and Canada and are the most practical means available for subtyping *S.* Enteritidis.[31] Pulsed-field gel electrophoresis (PFGE) of chromosomal digests shared through the PulseNet public health laboratory network has proven to be an extrordinarily useful tool in outbreak investigations and for answering specific epidemiologic questions, and now is the most common subtyping method used in the United States.[32]

The antibody response to human *Salmonella* infection has not been useful for serodiagnosis. *Salmonella* enterocolitis does not always provoke a measurable serological response, and the antibodies often cross-react with other bacteria. The Widal reaction, used historically to diagnose *S.* Typhi infection, measures agglutinating antibodies to O and H antigens of *S.* Typhi. The usefulness of this test has been severely hampered by nonspecific cross-reactivity with other *Salmonella* antigens, elevated titers due to nonspecific inflammatory disease, antibodies due to typhoid vaccination, and the suppression of O and H agglutinins by early treatment.[33] Testing for antibodies to the Vi antigen is useful for identifying typhoid carriers in epidemiological investigations (see Chapter 20).

4. Biological Characteristics of the Organism

Salmonellae are a hardy and resourceful group of organisms. They successfully parasitize a broad variety of hosts including insects, reptiles, amphibians, birds, and mammals. Although not true spore formers, they are resistant to drying and freezing and may survive for long periods in a nutrient-poor environment. The organism can survive for over 200 days in contaminated soil, 10 months in dust, more than 5 months in roach and rodent feces, and more than 4 years in dried whole egg. They multiply in food at temperatures ranging from 44 to 114°F (7–46°C) and can survive at a pH as low as 4.5.[34] Several *Salmonella* serotypes have been shown to survive quite well on the surface of fruits and vegetables[35], exploiting a possible conduit to the next colonized host.

Many salmonellae have life cycles in nature that guarantee long-term survival. Some have the ability to colonize specific loci in the gastrointestinal tract, tolerating the mild acidity of the animal's rumen or crop, or the bile in the gallbladder. Others have the ability to colonize the reproductive tracts of reptiles and birds, which means that the bacteria can infect the contents of eggs as they are formed, passing vertically to the next generation before it emerges from the shell; the evolution of this capacity well insulates such salmonellae from the environment.

4.1. Antimicrobial Resistance

The resistance of *Salmonella* to antimicrobial agents has been a matter of increasing concern. Antimicrobial resistance is important for clinical and public health reasons. Although antimicrobial treatment is not necessary in uncomplicated *Salmonella* enterocolitis and appears to prolong carriage,[36] antimicrobial treatment is essential in extraintestinal infections. The emergence of multiply resistant strains can lead to treatment failures in this setting and may require the use of new and more expensive antibiotics. Patients with antimicrobial-resistant *Salmonella* infections in FoodNet sites were more likely to have bacteremia and were hospitalized at a greater frequency and duration compared to patients with susceptible infections.[37] Antimicrobial resistance in *Salmonella* also has several important public health ramifications because simultaneous exposure to antimicrobials is associated with an increased risk of infection with resistant *Salmonella*. In one outbreak, 12 of 18 persons infected with the outbreak strain of multiply resistant *S.* Newport had taken a penicillin derivative in the week before the onset of their illness, compared to 0 of 11 persons infected with sensitive strains of *S.* Newport.[27] In most cases, illness began

within 3 days after taking the antibiotic, suggesting that the antibiotic provided a selective advantage for the resistant organism and converted asymptomatic colonization to overt infection. Exposure to antimicrobials can also lower the infectious dose for a resistant strain. This was demonstrated in a massive outbreak of multiply resistant *S.* Typhimurium infections in 1985.[38] In that outbreak, two brands of milk were contaminated, and the amount of implicated milk that persons reported drinking was an index of the dose of *Salmonella* they had received. Some persons affected by the outbreak had taken an antimicrobial to which the outbreak strain of *S.* Typhimurium was resistant just before becoming ill with salmonellosis. These persons reported drinking smaller volumes of implicated milk than ill persons not taking such antimicrobials, suggesting that a smaller dose of *Salmonella* caused illness in the former group.

At any given moment, some fraction of the population is taking antimicrobials for a variety of reasons. Because those persons appear to have heightened susceptibility to resistant strains of *Salmonella*, an outbreak due to a resistant strain may produce a somewhat larger number of cases than would an outbreak due to a sensitive strain. From 16 to 64% of cases in outbreaks of resistant *Salmonella* infections can be called "excess cases," occurring as a result of this interaction between resistance and prior or concurrent use of antimicrobials.[39] A more recent estimate of this included data from sporadic cases and suggested that 13–26% of salmonellosis cases could be attributed to the selective effect of taking an antimicrobial for other reasons.[40]

The overall level of antimicrobial resistance of *Salmonella* isolates nearly doubled from 1980 to 1990, though it has decreased somewhat since then. In the sentinel county survey of *Salmonella* isolates conducted by the CDC in 1989–1990, 31% of isolates were resistant to at least one antimicrobial,[19] a significant increase over the 16% resistance demonstrated in a similar survey in 1979–1980 (Table 1). The proportion of strains resistant to two or more agents rose from 12 to 25%. In 2004, 20% of isolates submitted to NARMS were resistant to at least one agent, 15% were resistant to two or more agents, while two strains resistant to five or more agents accounted for nearly 10% of all *Salmonella* isolates.[21] An *S.* Typhimurium strain commonly associated with definitive phage type 104 and resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline was 23% of all *S.* Typhimurium. Nearly 15% of all *S.* Newport isolates demonstrated resistance to nine antimicrobial agents, including extended spectrum cephalosporins.

Considerable evidence has accumulated about the major role of antimicrobial use in food animals that become a source of human infections with antimicrobial-resistant *Salmonella* in the United States.[41] In the 1984 sentinel county survey, the drugs to which *Salmonella* were likely to be resistant were frequently the ones that are seldom used in treating human infections, including streptomycin, nitrofurantoin, and kanamycin, yet these drugs are frequently used in agriculture and animal husbandry.[39] An increase in tetracycline resistance occurred despite the fact that the number of prescriptions dispensed for human use of

Table 1. Rates of Resistance to Specific Antimicrobials for Salmonellosis, Sentinel Country Surveys, 1979–1980, 1989–1990 and NARMS, 2004[a]

	Percent of isolates resistant		
Antimicrobial	1979–1980 ($n = 511$)	1989–1990 ($n = 484$)	2004 ($n = 1783$)
Ampicillim	8	14	12
Cephalexin	1.4	0.2	–
Chloramphenicol	0.8	3	7.6
Colistin	0	0	–
Gentamicin	0	4	1.3
Kanamycin	3.5	8	2.8
Nalidixic acid	0	0.2	2.6
Nitrofurantoin	1	1.0	–
Streptomycin	12	23	11.8
Sulfisoxazole	8	14	13.2
Tetracycline	8.6	24	13.5
Trimethoprim-sulfamethoxazole	0.2	0.4	1.8
Any agent	17	31	20.4
Two or more agents	12	25	15

[a]Adapted from MacDonald,[18] 1978–1980, Lee,[19] 1989–1990, NARMS annual report[21], 2004.

tetracycline decreased 61% between 1973 and 1984. During that period, tetracyclines continued to be used extensively as growth enhancers, particularly among cattle. More compelling evidence comes from outbreak investigations in which resistant strains have been traced from ill persons back through the food distribution chain to the farm. In one outbreak a multiply resistant strain of *S.* Newport was traced from ill persons in one state back through supermarket distribution and the meat processing plant to a farm where the beef cattle had been fed nontherapeutic doses of chlortetracycline.[27] In another outbreak, almost 1,000 persons in California became infected with a strain of chloramphenicol-resistant *S.* Newport with a unique plasmid profile. The chain of transmission was followed from ill persons, to hamburger, to beef from meat packers, to slaughterhouses, and, finally, to cows and calves on farms where chloramphenicol had been used.[29] At each stage, the unique epidemic strain of *S.* Newport was isolated. More recently, the emergence of a highly resistant strain of *S.* Newport infections in the Northeastern United States was heralded by outbreaks on dairy farms.[42] Experience in other countries suggest that decreased use of antimicrobials in animal feed may result in a decrease in antimicrobial-resistant strains of *Salmonella* causing human infection.[43]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Between the inception of national laboratory-based *Salmonella* surveillance in 1962 and 1976, the incidence of reported *Salmonella* isolations in humans increased slightly. Between 1976 and 1986, the incidence rose by more than 60%, from 10.6/100,000 in 1976 to 17.4/100,000 in 1986, declined to 15/100,000 in 1994, and was 11 per 100,000 in 2005. This overall trend includes a sharp peak in *S.* Typhimurium infections related to a massive milk-associated outbreak in 1985 with more than 16,000 culture-confirmed cases, a dramatic increase in the isolation rate of *S.* Enteritidis in the 1980s and 1990s, and an increase and subsequent decrease in *S.* Heidelberg (Figure 2). This increase in reported isolates was paralleled by an increase in clinical cases reported to the *Morbidity and Mortality Weekly Report* during the same period (Figure 1). The actual number of infections occurring annually is likely to be more than 1.4 million, the vast majority of which are mild and undiagnosed (see Section 3.2). In FoodNet, the incidence of *Salmonella* infections has not changed significantly from the baseline period of 1996–1998 to 2006.[44] The top six serotypes in FoodNet sites showed disparate trends during this period, with the estimated incidence of

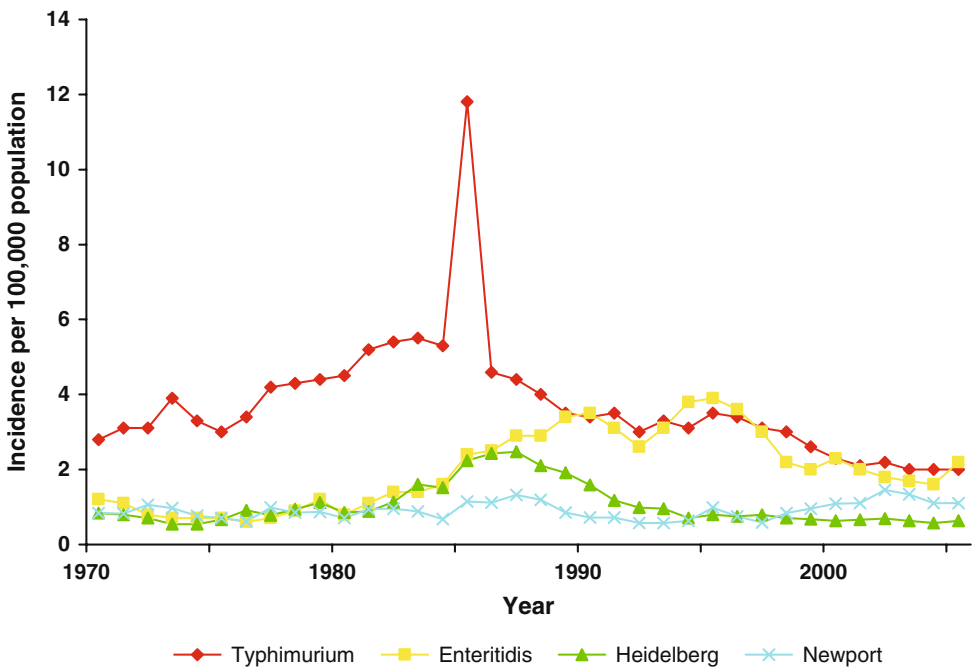


Figure 2. Isolation rates for selected serotypes of *Salmonella* in the United States from 1970 to 2005. From the CDC[51] (1985 peak for *S.* Typhimurium includes reported isolates from massive outbreak with >16,000 culture-confirmed infections.[38]).

Salmonella Javiana, *S*. Newport, and *S.* Enteritidis increasing, *S*. Heidelberg and *Salmonella* Montivideo not changing, and only *S. Typhimurium* decreasing.

The epidemiology of *Salmonella* infection varies considerably by serotype. *Salmonella* Typhimurium accounts for 15–25% of human isolates and comes from a variety of different sources, including meat, poultry, and eggs. *Salmonella* 4,5, 12:i:-, a monophasic serotype related to*S.* Typhimurium, has emerged as an important cause of human salmonellosis, first in Europe as a multidrug-resistant strain associated with pork[45] and more recently as a susceptible strain in the United States, with a possible poultry reservoir.[46] *Salmonella* Enteritidis remains a dominant serotype in the United States and many other industrialized nations.[47,48] This serotype is strongly egg associated: between 1985 and 1991, 82% of *S.* Enteritidis outbreaks with known vehicle were egg associated and the same was true for sporadic infections, though now consumption of poultry is also an important source of this serotype.[49–51] The 10 most common serotypes account for 66% of infections. Five of these are also among the top 10 reported from nonhuman sources (Table 2).[52] *Salmonella* Newport has an important bovine reservoir. *Salmonella* Heidelberg and *S*. Montevideo appear to have poultry reservoirs. *Salmonella* Javiana is increasing in frequency, has caused outbreaks related to produce, and may have a reservoir in amphibians.[53, 54]

The general increase in the incidence of salmonellosis in the United States after 1950 parallels similar increases seen in many industrialized countries. Several factors contributed to the increase, including changes in methods of raising food animals intensively and shipping them to slaughter and the increased mechanization of animal slaughter. Changes in the cooking practices of the population are also of concern, including a greater reliance on precooked convenience foods, on foods eaten outside of the home, and a decline in what children learn about cooking at home and in school. The more recent decrease in salmonellosis may reflect greater attention to contamination control in animal husbandry and slaughter, as well as efforts to improve food-handling practices.

The prevalence of *Salmonella* carriage among the general population is not known with accuracy in the United States, but has been estimated to be between 0.15 and 0.2%.[16,55]

5.2. Epidemic Behavior and Contagiousness

Most cases of human salmonellosis result from contaminated food, although infection has been transmitted by an enormous variety of other vehicles (see Section 6). Cases may occur as part of recognized outbreaks but the majority occur as sporadic cases.

Outbreaks of salmonellosis that are reported to the CDC account for less than 10% of reported isolates in most years. In 1998–2002, the mean size of reported outbreaks of *Salmonella* infections was 28 persons, suggesting that only relatively large outbreaks were identified and reported.[12] In the 1979–1980 sentinel county survey, only 21% of reported isolates were associated with outbreaks or linked to other cases reported to local health departments. The remaining cases were not associated with other cases or recognized outbreaks and are considered "sporadic."[17] Case–control studies of sporadic cases may relate illness to the same exposures as outbreaks, and in some cases it has been possible to link many "sporadic" cases to a common source.[28,50]

Salmonella outbreaks often have distinctive patterns. Most distinctive is the explosive outbreak from a common source of exposure, dramatically exemplified by the

Table 2. The Ten Most Common Serotypes Identified Among Human *Salmonella* Infections and Nonhuman Sources in 2005[a]

Human			Nonhuman		
Serotype	No. of isolate	%	Serotype	No. of isolate	%
Typhimurium	6982	19.3	Typhimurium	2230	19.0
Enteritidis	6730	18.6	Newport	1254	10.7
Newport	3295	9.1	Heidelberg	887	7.5
Heidelberg	1903	5.3	Senftenberg	646	5.5
Javiana	1324	3.7	Agona	437	3.7
I 4,[5],12:i:-	822	2.3	Derby	418	3.5
Montevideo	809	2.2	Hadar	413	3.5
Muenchen	733	2.0	Kentucky	381	3.2
Saintpaul	683	1.9	Montevideo	276	2.3
Braenderup	603	1.7	Meunchen	252	2.2
Other	12300	34.0	Other	4518	39.0
Total	36184			11712	

[a]From the CDC.[51]

massive milkborne outbreak in Illinois in 1985 involving more than 16,000 reported cases.[38] A pattern more difficult to recognize is the prolonged epidemic curve associated with continued exposure to a widely distributed contaminated food source or product, such as the outbreaks due to *S.* Newport-contaminated roast beef in several northeastern states,[28] *Salmonella* Eastborne in contaminated chocolate,[56] *Salmonella* Newbrunswick in powdered milk,[57] marijuana contaminated with *Salmonella* Muenchen,[30] or *Salmonella* Tennessee in peanut butter.[58] These outbreaks are most easily recognized when they involve an unusual serotype. Molecular subtyping of *Salmonella* isolates by PFGE in state public health laboratories and comparison of similar patterns through the PulseNet laboratory network have allowed for detection of widespread outbreaks among more common serotypes, such as *S.* Typhimurium due to contaminated ground beef[59] and *S.* Newport associated with tomatoes.[60] It is likely, though, that many small foodborne outbreaks occur in families, and this has led to difficulty determining the relative importance of foodborne versus intrafamilial spread. In one study, 39% of cases of salmonellosis in infants were preceded by gastroenteritis in an adult family member, suggesting that intrafamilial spread to infants may follow salmonellosis in other family members.[61] In general, sustained person-to-person transmission of salmonellosis is unlikely (see Section 5.8).

5.3. Geographic Distribution

Nontyphoidal salmonellosis appears to be predominantly a disease of industrialized nations and industrialized agriculture. Worldwide, the proportion of *Salmonella* infections due to nontyphoid serotypes of *Salmonella* appears to increase with the degree of economic development, while the proportion due to *S.* Typhi declines (Figure 3).

Isolation rates vary considerably across the United States. Differences in reported isolation rates between states must be interpreted with caution since it has been shown that isolation rates correlate with per capita state laboratory expenditure and with the number of stool cultures submitted to the state laboratory.[62] Consistent regional differences exist, however, with the highest rates observed in New England, Middle Atlantic, and Pacific states and the lowest rates in the West and West–North Central states, suggesting that real variations may exist in *Salmonella* exposure.

Within the United States, considerable geographic variation exists among serotypes and over time. For example, *S.* Enteritidis has been particularly common in the northeastern part of the United States. Though it has recently declined, it often greatly exceeded the number of *S.* Typhimurium isolates there in that region. *Salmonella* Enteritidis increased rapidly in the West in the 1990s, where *S.* Newport and *Salmonella* Dublin have long been concentrated (Figure 4). *Salmonella* Javiana is associated

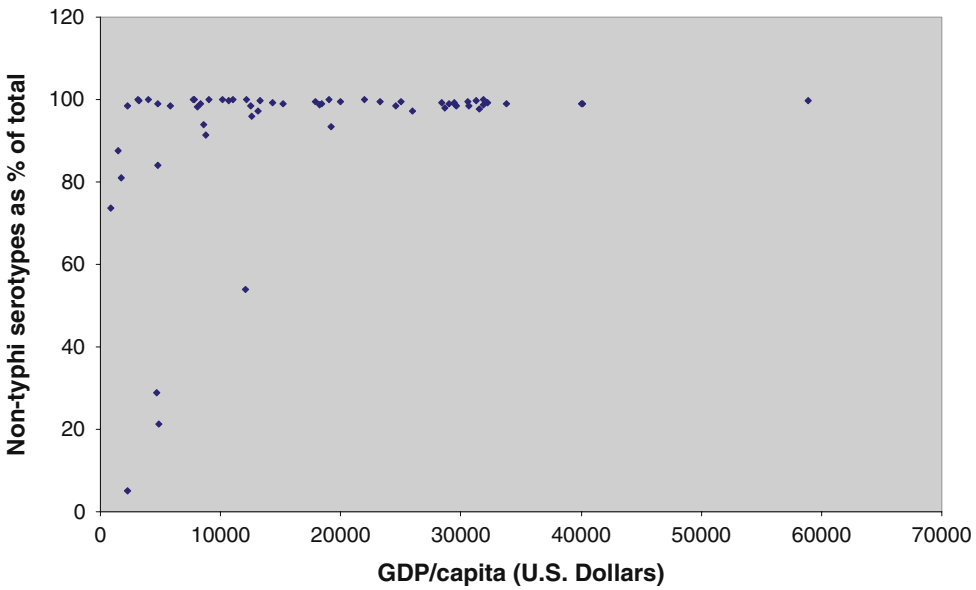


Figure 3. Nontyphoid serotypes as a percentage of all *Salmonella* isolates (including *S.* Typhi) as a function of per capita gross national product, showing the increasing importance of nontyphoidal salmonellosis with increased economic development. Data from Global *Salmonella* Surveillance country databank accessed via the World Wide Web at http://www.who.int/salmsurv/activities/databank/en/ and the United States Central Intelligence Agency CIA World Fact Book accessed via the World Wide Web at https://www.cia.gov/library/publications/the-world-factbook/.

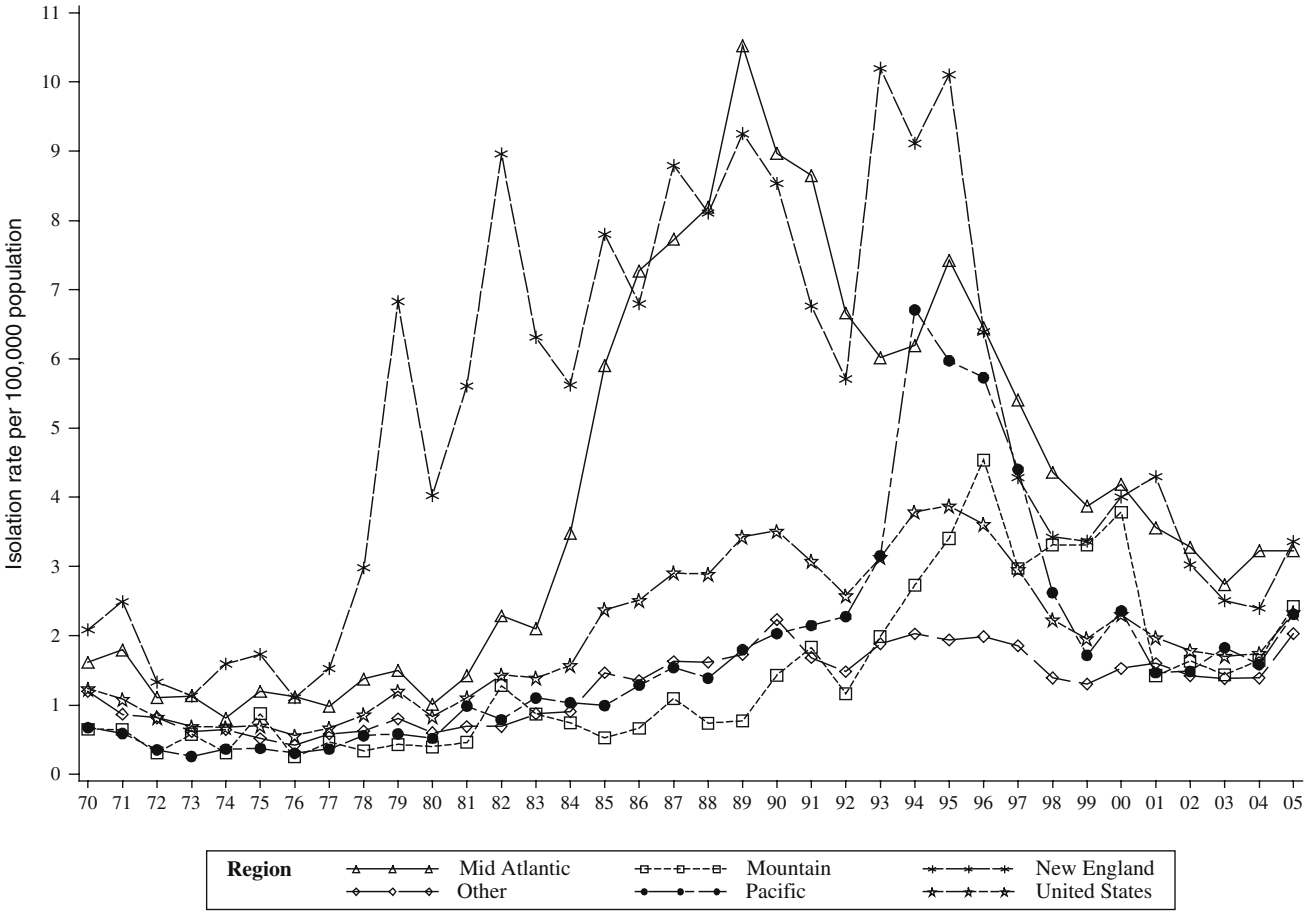


Figure 4. Annual isolation rate of *S.* Enteritidis, United States, 1970–2005, by region. From the CDC.[51]

with the Southeast, S. Oranienberg with the Southwest, and S. *Weltevreden* with Hawaii.[3]

5.4. Temporal Distribution

Salmonella isolation shows a consistent seasonal pattern, with a summer and fall peak. This seasonal pattern is likely the result of seasonal variation in levels of infection of animals and in the resulting contamination of foods, as well as from the more rapid growth of *Salmonella* at warmer temperatures. The amplitude of the seasonal variation varies among the different serotypes.

5.5. Age and Sex

The age- and sex-specific isolation rates for *Salmonella* is shown in Figure 5. The isolation rate is highest in the first year of life, peaking at more than 160 cases per 100,000 population per year in the second month of life.[63] The rate of infection declines rapidly among individuals 10–19 years old and then begins to rise among those older than 60. Some degree of detection bias probably exists, since infants and the elderly may be more likely to have stool cultures performed when they are diagnosed with gastrointestinal infection. Nonetheless, this pattern probably reflects real age-specific differences in host susceptibility and in exposure rates. Substantial differences exist in the age specificity among the various serotypes, which are poorly understood but which presumably reflect differences in vehicle of transmission or of underlying serotype-specific immunity.

5.6. Race

Data on race are not routinely collected in national laboratory-based surveillance and no national data on the

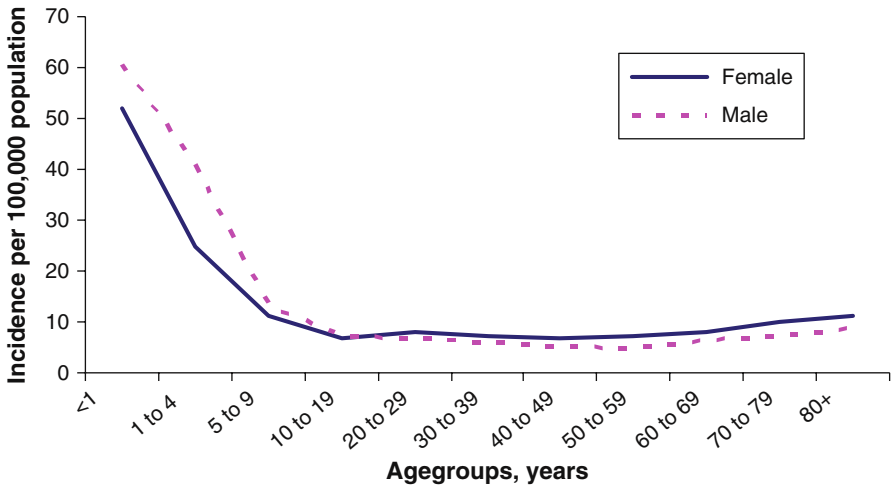


Figure 5. *Salmonella* isolation rates by age and sex, per 100,000 population, United States, 2005. From the CDC.[51]

relative isolation rates by race have been reported. For unknown reasons, the risk that a *Salmonella* infection will be caused by a resistant strain is higher among black than white infants.[19]

5.7. Occupation

Few data are available to demonstrate that high-risk occupations for salmonellosis exist. Some studies have found higher rates of *Salmonella* carriage among food handlers, which may reflect their high level of contact with raw food and the frequency with which they are cultured.[64] In the past, sewage workers were considered to be at high risk of *Salmonella* infections, particularly typhoid fever, but existing evidence does not suggest that they are currently at increased risk.[65]

5.8. Occurrence in Different Settings

Salmonella outbreaks may create particular problems at institutions, including schools, prisons, nursing homes, hospitals, mental hospitals, and institutions for the developmentally disabled. Between 1963 and 1972, 28% of all *Salmonella* outbreaks reported to the CDC occurred in institutions; two thirds of these occurred in acute-care hospitals, pediatric wards, and newborn nurseries.[66] Institutional outbreaks tend to be more serious than those in other settings; the case-fatality rate for all institutional outbreaks between 1963 and 1972 was 2.3% and for those occurring in nursing homes, 8.7%. In the past, nosocomial outbreaks have occurred due to the use of contaminated medical and pharmaceutical products including carmine dye, pancreatic enzymes, platelets, and extracts of thyroid, adrenal cortex, pancreas, and pituitary.[66] Other important hospital outbreaks have been caused by contaminated foods, including powdered eggs, dried milk, and human breast milk.[67] The number of nosocomial *Salmonella* outbreaks reported to the CDC has decreased in recent years, but outbreaks due to *S.* Enteritidis in shell eggs have occurred in nursing homes and hospitals, reflecting the importance of eggs as the vehicle of *S.* Enteritidis.[46] Among 52 foodborne outbreaks of known etiology in nursing homes reported to the CDC between 1975 and 1987, 27 (52%) were caused by *Salmonella*, particularly *S.* Enteritidis.[68] *Salmonella* accounted for 81% of deaths associated with these outbreaks.

Although person-to-person spread is frequently suspected in institutional outbreaks of *Salmonella*, it has been rigorously demonstrated in relatively few. Most recent institutional outbreaks are caused by food products of animal origin or contaminated produce. The risk of secondary transmission within hospitals probably varies according to the patient population and setting. Transmission is particularly likely to occur in neonatal nurseries, where peripartum transmission may result in an infected infant and the high level of nursing intervention and the susceptibility of neonates to *Salmonella* predispose to secondary transmission. On regular hospital wards the risk may be considerably less. No secondary transmission was documented in a hospital outbreak involving more than 200 nurses who developed salmonellosis after a "nurse appreciation day" meal.[69] Infection control measures included emphasis on hand washing and exclusion of nurses with enterocolitis until symptoms resolved; negative stool cultures before returning to duty were only required for those working in the neonatal

or adult intensive care unit, nursery, or renal dialysis unit. However, transmission to a patient did follow an outbreak of illness among family practice residents, emphasizing the need for vigilance and rigor in basic prevention.[70]

5.9. *Salmonella* Infections in the Compromised Host

A variety of conditions have been associated with increased risk of *Salmonella* infections, including gastric surgery, decreased gastric acidity, sickle-cell anemia, lymphoma, Hodgkin's disease, metastatic cancer, and infection with human immunodeficiency virus (HIV).[55,71,72] These are related to different mechanisms of defense against *Salmonella* infections (see Section 7). Patients with sickle-cell anemia and other diseases that compromise the reticuloendothelial system have an increased rate of *Salmonella* bacteremia and *Salmonella* osteomyelitis. The decreased ability to clear opsonized bacteria of these patients and perhaps the increased availability of iron may play a major role.[73] Patients with neoplastic disease are also at increased risk of salmonellosis, and lympho-proliferative disorders appear to be more strongly associated with salmonellosis than are solid tumors[72] (see Section 8).

5.10. *Salmonella* Infections and AIDS

Salmonella has emerged as an important pathogen among patients with acquired immunodeficiency syndrome (AIDS). In a San Francisco study, the annual incidence of salmonellosis among patients with AIDS was almost 20 times higher than that in men in a similar age group who did not have AIDS.[71] The proportion of bacteremic *Salmonella* infections is also increased in patients with AIDS; *S.* Typhimurium and *S*. Dublin were more likely to cause bacteremia among these patients than among patients without HIV infection. *Salmonella* bacteremia in patients with AIDS often occurs in the absence of an identifiable episode of enterocolitis and frequently recurs after appropriate antimicrobial treatment.[74] Recurrent *Salmonella* bacteremia was made an indicator disease in the AIDS case definition in 1987. *Salmonella* bacteremia in HIV-infected individuals may precede and foreshadow the development of AIDS; in one-third of the patients in the San Francisco study, salmonellosis occurred shortly before they were diagnosed to have AIDS.[71] Increases in *Salmonella* bacteremia, particularly caused by *S.* Enteritidis and *S.* Typhimurium, mirror the demography of the AIDS epidemic.[75,76] *Salmonella* infections represent a large and potentially preventable source of mortality in this group. Patient education and the avoidance of high-risk foods have an important role in prevention.

6. Mechanisms and Routes of Transmission

Human infections are often related directly or indirectly to infection in animals. Transmission can occur via a variety of specific routes, including foodborne, waterborne, animal-to-person contact, person-to-person contact (either direct or indirect), and transfusion. However, up to 95% of infections with *Salmonella* in the United States are foodborne in origin.[77] Data on the sources of foodborne salmonellosis outbreaks indicate that foods of animal origin, especially poultry products, are the most common sources (Table 3). Produce items caused more than 10% of recent salmonellosis outbreaks, consistent with a trend toward more outbreaks of all etiologies due to fresh produce.[78] The typical foodborne outbreak is the result of at least two food-handling errors: one that permits the contamination to occur or persist and another that permits sufficient bacterial growth to reach the infectious dose.

The epidemiological complexity of tracing the sources of specific contaminated foods can be challenging. A few serotypes are associated with a specific vehicle. For instance, *S*. Dublin infection is strongly associated with the consumption of raw milk and *S.* Enteritidis with undercooked eggs.[47,79] Infection with a multidrug-resistant strain of *S*. Newport has been linked to beef in both outbreak and sporadic case–control studies.[80 81] When a convenient bacteriologic strain marker is available, it has been possible to retrace the chain of transmission to farms of origin.[27,29] If the serotype is rare, or the molecular subtype by PFGE is available, it may be possible to trace it even further back. For instance, the international epidemic of *Salmonella* Agona infections was traced to Peruvian fish meal, used in poultry

Table 3. Foodborne Outbreaks of Salmonellosis Reported 1998–2002, by Impilcated Vehicle[a]

Food vehicle	No. of outbreaks	Percent of outbreaks of known vehicle
Eggs	57	15
Poultry	50	13
Vegetable	25	7
Fruits and nuts	18	5
Pork	17	5
Dairy	11	3
Beef	9	2
Finfish/Shellfish	6	2
Other foods	6	2
Multiple foods	177	47
Known	376	
Unknown	209	
Total	585	

[a] Adapted from Lynch.[12]

feed, that introduced this formerly rare serotype to several continents simultaneously, where it has since persisted in poultry.[82] In the United States, several serotypes have been associated increasingly, though not exclusively, with particular fruit and vegetable food vehicles, including widespread outbreaks of *S.* Javiana and *S*. Newport infections due to domestic tomatoes and recurrent outbreaks of *Salmonella* Poona infections from imported cantaloupes.[83,84] Recurrent outbreaks due to one PFGE-defined strain of *S*. Newport associated with tomatoes have been traced to farms in one state by isolation of the outbreak strain in the growing environment.[60] *Salmonella* can be introduced and perpetuated on a farm through contaminated feeds, the introduction of infected stock, or by wild animals.

Foods may be contaminated in different ways: meat by contents of the gastrointestinal tract during the slaughter process or in vivo if the animal was bacteremic at the time of slaughter; milk by feces during the milking process or by mastitis in the milked cow; prepared foods during processing by the use of contaminated ingredients or by cross-contamination within the processing plant itself. In the kitchen, food may be cross-contaminated from raw foods or other sources. Once introduced, *Salmonella* can survive and multiply in many different foods, including meats, dairy products, and produce. Although food handlers are often found to be infected in the course of investigating a foodborne outbreak investigation, they are most often infected because of their exposure to contaminated foods and are less commonly shown to be the source of the contamination themselves.[55] Food may be a source of *Salmonella* even when not consumed. In a recent FoodNet case–control study of salmonellosis in infants, the identified risk factors included having ridden in grocery cart next to meat or poultry.[85]

In addition to foodborne transmission, large waterborne outbreaks of salmonellosis have occurred, when drinking water supplies have been contaminated in the absence of proper chlorination.[86,87]

Salmonella can reach an individual from animal reservoirs via a variety of other routes. Persons in direct contact with ill animals may become infected themselves. Pet turtles, which were almost universally colonized with *Salmonella*, emerged as an important source of the disease in the United States in the early 1970s.[88] Pet iguanas and other reptiles are now an important source of salmonellosis.[88] Chameleons, aquarium fish, chicks, and other potential pets may also be colonized with *Salmonella* and may occasionally be sources of infection.[90,91] Marijuana contaminated with *S.* Muenchen was the source of an interstate outbreak probably due to the presence of animal feces.[30] A series of outbreaks of *Salmonella* Cubana infections was traced to carmine dye made from powdered insects that was used as a marker of gastrointestinal motility.[92] An outbreak of *Salmonella* Arizona infections was traced to powdered rattlesnake capsules sold as a folk remedy.[93] Multidrug-resistant *S.* Typhimurium was transmitted by contact with hamsters sold as "pocket pets."[94]

Salmonellosis can also be transmitted from one infected person to another, particularly among a group of unusually susceptible individuals (see Section 5.2). Foodborne outbreaks can be linked to infected food handlers, who may sometimes be the source; often the food handler was actually infected by the food.[95] Nosocomial outbreaks of salmonellosis that are not foodborne typically occur in the nursery, and transmission from one child to the next occurs on the unwashed hands of the healthcare worker or via medical equipment. Many other routes of nosocomial transmission are possible. Infection can follow transfer of the organism via endoscope from one patient to the next.[23] *Salmonella* Kottbus has been transmitted through unpasteurized human milk, collected for infants at a human milk bank, from a woman with asymptomatic mammary carriage.[96] *Salmonella* Choleraesuis was transmitted through platelet transfusions after the platelets were collected from a bacteremic donor.[97] Infection has been transmitted from soiled patient bedclothes to hospital laundry workers.[98]

Although epidemiological data from developing countries are limited, transmission of salmonellosis in those countries may include all of the above routes. Nosocomial reservoirs may be of greater importance than in industrialized countries, particularly for multiply resistant and invasive strains, such as those described in Brazil, Rwanda, and the Ivory Coast.[99–101]

7. Pathogenesis and Immunity

The incubation period after ingestion of *Salmonella* usually ranges from 12 to 48 h, though longer incubation periods have been documented[102]. The pathogenesis of *Salmonella* infections is incompletely understood. The multiplicity of serotypes and animal hosts involved introduces much complexity; a serotype that is nonpathogenic in one animal species may produce severe illness in another. Laboratory models need to be interpreted with caution. For instance, *S.* Typhimurium infections in the mouse may be more analogous to *S.* Typhi than to *S.* Typhimurium infections in humans. The clinical expression of infection is affected by serotype and strain of the bacteria; the status of the underlying health and gut flora of the host; and the dose, vehicle, and route of transmission.

Following ingestion, the organisms traverse the gastric acid barrier and multiply in the small intestine. The bacteria penetrate and damage the intestinal mucosa, are ingested by macrophages, and may multiply in a limited fashion in mesenteric lymphoid tissues. In severe invasive infection, bacteremia and focal infections in distant tissues occur.

After more than a century of study, the virulence properties of these organisms remain incompletely understood. Many potential factors and mechanisms have been described, which may be variably present in different serotypes and strains. Some have been well characterized and undoubtedly more remain to be discovered. The O-specific side chain portion of lipopolysaccharide (LPS) is associated with invasiveness, and rough mutants with incomplete O-side chains have markedly diminished virulence.[103] The lipid A portion of LPS is responsible for the endotoxin-mediated effects of the *Salmonella* infection.[104]

The process includes several distinct steps, no single one of which explains the entire pathogenic spectrum: (1) Attachment and penetration. Adhesins facilitate the attachment of salmonellae to intestinal mucosal cells and may be necessary for mucosal invasion.[105] *Salmonella* can induce ordinarily nonphagocytic mucosal cells to engulf them, an unusual ability that gives them rapid access to protected intracellular sites.[106] (2) Inflammation and tissue destruction. The fever, diarrhea, and evidence of colitis that often accompany salmonellosis may be the result of local inflammation, effects of bacterial endotoxin, or of specific cytotoxins that cause mucosal cell death.[107] (3) Intracellular multiplication in macrophages. Lethality in the mouse model is associated with the ability to multiply in macrophages, which may be a function of the presence of flagella, plasmid-associated factors, and an ability to slow acidification within the macrophage.[108–110]

The outcome of infections is partly dependent on the number of ingested bacteria that reach the intestine. Small numbers of ingested *Salmonella* may cause transient asymptomatic intestinal colonization, while larger doses of the same strain may produce overwhelming or even fatal illness. The minimum dose necessary to cause illness varies with the serotype, the vehicle, and a variety of host factors. Although early volunteer studies indicated the infectious dose ranged from 10^5 to 10^{10} organisms, the minimal dose that causes illness in some circumstances may be substantially lower, even as low as 10 organisms.[111]

There are several major barriers in the host's defense against *Salmonella* infection. The first is gastric acidity, which can reduce the number of viable organisms by several logs within minutes. The degree of protection provided by gastric acid depends on the vehicle of infection; *Salmonella* may traverse the stomach more rapidly when consumed in liquids than in solid foods, and contaminated foods that buffer gastric acid well, such as chocolate, cheese, or milk, may transmit infection more efficiently than foods that do not. Persons with more neutral gastric pH, such as the elderly, those taking antacids or other antiulcer medications, and those who have had gastric surgery, are at increased risk for infection.[17,55]

The normal intestinal flora are a second major barrier to infection with salmonellosis. Disruption of the flora, particularly with antimicrobials that are active against the anaerobic flora such as penicillin or tetracycline, decreases the infectious dose needed to produce illness.[38,112] If the infecting strain is resistant to the antimicrobial used, therapy for an unrelated condition may convert a silent transient colonization to overt clinical infection, when the normal flora are suppressed.[27,113] Cellular immunity is the third major defense against bacteremia and invasive illness. This can be overcome in the normal host by certain highly invasive serotypes, such as *S.* Choleraesuis, or *S.* Paratyphi, or by very large doses of other serotypes.[114] Hemolytic conditions, such as sickle-cell disease, bartonellosis, or malaria, predispose to invasive infection.[115] Persons with defective cellular immunity, such as those receiving immunosuppressive therapy, with advanced malignancies, or infected with HIV, are also at increased risk for invasive illness (see Sections 5.9 and 5.10).

Immunity to *Salmonella* infection is partial at best. In volunteer experiments, patients rechallenged with the homologous strain became ill a second time, although with somewhat milder symptoms than on initial exposure.[116] In animal models, experimental vaccines can prevent death, but not infection. A vaccine against *S.* Choleraesuis, an economically important pathogen in swine, has now been licensed for use in swine.[117]

The humoral response of humans to experimental infection has been defined. Agglutinating antibodies develop against the somatic (O) and flagellar (H) antigens, even following asymptomatic infections.[118] These antibodies do not always appear, they wane after several months, and sufficient cross-reactions occur with other enteric bacteria that they have been of little diagnostic or epidemiological utility. The nature of the cellular immune response to salmonellosis in humans has not been clearly defined.

8. Patterns of Host Response

The host response to *Salmonella* infection includes asymptomatic colonization, gastroenteritis, focal soft tissue infection, meningitis, bacteremia, sepsis, and death. The pattern of host response may be dictated by the virulence of the specific strain, the size of the inoculum, and specific host

factors including age, gastric acidity, immunologic competence, prior antimicrobial exposure, and specific illnesses. Disorders of the gastrointestinal tract (gastrectomy, achlorhydria, esophageal, gastric, or intestinal malignancies) may predispose to enterocolitis, and defects of cellular immunity (leukemia, lymphoma, HIV infection) or reticuloendothelial cell function (sickle-cell anemia, bartonellosis, malaria) may predispose to bacteremia and invasive disease.[55,72] Age is also an important determinant of host response. Bacteriuria, and frank urinary tract infection with *Salmonella,* is more frequent among older women, and is associated with specific serotypes, such as *S.* Tennessee.[119] The incidence of salmonellosis is higher among infants and the elderly and the frequency of bacteremia is higher in both of these age groups.[120] The mortality of *Salmonella* infection is also considerably higher at the extremes of age, as demonstrated by outbreaks in nurseries and nursing homes.[66]

After infection occurs, the organism persists in the gut for a median of 5 weeks, even after all symptoms have ceased. This convalescent carriage can be prolonged in young children.[121] Paradoxically, treatment with antimicrobials does not shorten the duration of carriage, but typically prolongs it, suggesting that the final resolution of infection may depend on the competitive effects of other gut bacteria as well as on the immune response of the host.[36,122] In persons of the HLA-B27 genotype, acute salmonellosis can trigger the reactive arthropathy, Reiter's syndrome, leading to prolonged and recurrent episodes of joint pain, urethritis, and uveitis.[123]

The most important determinant of host response may be the organism itself. Certain serotypes appear to be unusually virulent and are isolated more often from the blood and CSF relative to their overall isolation rate. *Salmonella* Choleraesuis, *S.* Dublin, and *S.* Paratyphi A, B, and C are more commonly isolated from blood, and *Salmonella* Panama, *Salmonella* Heidelberg, and *Salmonella* Saintpaul are disproportionately common isolates from CSF.[124,125] Some serotypes, such as *S.* Tennessee, are disproportionately common in urine specimens, particularly in older women, and may be uropathogenic.[119]

8.1. Clinical Features

The clinical manifestations of infection with nontyphoidal *Salmonella* can be grouped into several clinical syndromes with some overlap.

Acute enterocolitis, or gastroenteritis, is the most common syndrome of *Salmonella* infection. Initial symptoms often include nausea, vomiting, and headache, followed by abdominal cramps and diarrhea, which are the predominant manifestations. The stools are normally loose and of moderate volume; blood is occasionally seen. Voluminous, cholera-like diarrhea has been reported, as have small-volume, dysenteric stools associated with tenesmus, but these are unusual manifestations. Fever, often accompanied by chills, is present in about 50% of patients. Diarrhea usually lasts 3–7 days, but may be prolonged.[55] The larger the dose of organisms ingested, the shorter the incubation period and the more likely the patient is to have fever, vomiting, and a more severe illness in general.[126]

The enteric fever syndrome is classically associated with *S.* Typhi, but may at times occur with other serotypes. This syndrome is characterized by a prolonged course with prominent and sustained fever, anorexia, abdominal pain, splenomegaly, and headache. Respiratory symptoms may be prominent and hepatomegaly, neurological manifestations, and relative bradycardia may occur in nontyphoidal enteric fever. Complications include intestinal hemorrhage and perforation, myocarditis, seizures, and localized infections, but are less common than in enteric fever due to *S.* Typhi.

Bacteremia without enteric fever may also occur due to nontyphoidal *Salmonella*. In children, "silent" bacteremia during enterocolitis may occur in 5–10% of patients,[127] but *Salmonella* bacteremia with high fever may occur without other signs of enterocolitis or enteric fever, particularly among persons with AIDS. Localized extraintestinal infections may result. Endarteritis, particularly infection of atherosclerotic aortic aneurysms, may occur in patients with preexisting atherosclerotic disease. Endocarditis is rare, and usually occurs on abnormal valves; infection of prosthetic heart valves has also been reported. *Salmonella* Choleraesuis is responsible for a disproportionate number of endothelial infections. These infections can rarely be cured by antibiotics alone and surgical intervention is often necessary.[55] *Salmonella* meningitis occurs primarily in infants, and even with adequate antibiotic therapy, the mortality is high. Osteomyelitis and arthritis due to *Salmonella* may occur in normal hosts but are more common in immunocompromised persons, particularly those with hemolytic disorders. *Salmonella* is the most common cause of osteomyelitis in patients with sickle-cell anemia. Other complications of *Salmonella* bacteremia include pneumonia, empyema, endocarditis, intracranial abscess, urinary tract infections, and abscesses of the spleen, liver, and other soft tissue.[115]

8.2. Diagnosis

The diagnosis of *Salmonella* enterocolitis is made by stool culture. The diagnosis should be considered in any patient with moderate-to-severe gastroenteritis; the presence

of headache or fever makes the possibility of this disease more likely. Fecal leukocytes may be present, but are not diagnostic. The differential diagnosis of *Salmonella* enterocolitis includes viral gastroenteritis and other bacterial enteric infections, including those caused by *Shigella*, enterotoxigenic, enterohemorrhagic, and enteropathogenic *Escherichia coli, Campylobacter* species, *Vibrio* species, and *Yersinia enterocolitica*. The disorder must also be differentiated from toxin-mediated foodborne illnesses including those caused by *Staphylococcus aureus, Bacillus cereus*, and *Clostridium perfringens*. Compared to patients with these toxin-mediated disorders, persons with salmonellosis usually have less prominent vomiting and are more likely to have prolonged diarrhea and fever. The shorter incubation periods of toxin-mediated foodborne illness can be an important clue to distinguish them from salmonellosis. The presence of frequent, small-volume, bloody or mucus-streaked stools, tenesmus, and large numbers of fecal leukocytes makes campylobacterosis or shigellosis more likely than salmonellosis. A history of ingestion of raw milk, undercooked eggs or meat, or exposure to lizards or farm animals within the 48 h before onset of illness may suggest the diagnosis of salmonellosis. Ultimately, however, the diagnosis depends on cultures of stool, or in the case of high fever or invasive disease, blood, CSF, or other body fluids.

9. Control and Prevention

The control of salmonellosis depends on understanding the routes and cycles of transmission well enough to interrupt them. Control of foodborne salmonellosis can occur at many levels, at the farm, during truck transport, the slaughter plant, or food production facility, to the cook and consumer. Once the transmission is understood and the critical control points are understood, intervention can be highly successful.

Interventions at the level of the farm can succeed. In Scandinavia, the prevalence of salmonellae in chicken flocks has been greatly reduced by exposing the chicks to normal fecal flora shortly after they hatch, thus increasing their resistance to subsequent colonization with *Salmonella*.[128] In many countries, including the United States, a serotype that is highly pathogenic to poultry—*S*. Gallinarum—has been virtually eradicated by a vigorous flock- and hatchery-based program of serological testing and destruction of infected birds.[129] In Denmark, a cost-effective program of testing swine and eggs for serologic evidence of infections, and elimination of infected flocks or swine herds has greatly reduced the frequency of contamination of those food products.[130]

Animals entering the slaughter plant may be free of *Salmonella*, may have silent colonization, or may be ill or even moribund with systemic salmonellosis. Visual inspection methods will detect some of the last group; the proportion of slaughtered cattle that are moribund correlates with the level of carcass contamination.[29] However, visual inspection cannot detect silent colonization, which is far more common. Slaughter and inspection safety practices based on the Hazard Analysis – Critical Control Point (HACCP) method were introduced in the late 1990s, and include performance measures based on the frequency with which *Salmonella* is isolated from the final product.[131] Condemned carcasses are sold for pet food or are sent to rendering plants to be converted to meal used to make animal feed. Unfortunately, the rendered product is often recontaminated with *Salmonella* before it leaves the rendering plant, so that animal feed in the United States is often contaminated with *Salmonella*. Many countries, but not the United States, require the routine pasteurization of animal feeds to interrupt this cycle.[132]

Salmonellosis related to fresh fruits and vegetables can be controlled if the level of contamination occurring in the field and during processing can be reduced. Strategies include using uncontaminated sources of water for application to edible portions of the plant, providing adequate sanitary facilities for field workers, washing fruits and vegetables after harvest in carefully disinfected water, shipping them on ice made from disinfected water, and not cutting or slicing them until immediately before consumption. Guidelines for good agricultural practices have been proffered[133] but implementation of industry-wide food safety programs for specific produce commodities has been challenging. For instance, raw sprout-related outbreaks continue to be a problem; some due to incomplete adherence to recommended sprout disinfection and testing protocols.[134]

Changes in food production practices can increase or decrease the likelihood of contamination of the finished product. For example, the pasteurization of milk protects the consumer against many raw-milk-associated pathogens, including *Salmonella*. Because of documented risk of salmonellosis and campylobacteriosis, the interstate sale of raw milk was banned in 1987. Outbreaks related to contaminated fruit juices decreased following implementation of heat treatment as part of a juice HACCP program.[135] In a series of *S*. Newport outbreaks traced to precooked roast beef in the late 1970s, the cooking process was found to be inadequate to kill *Salmonella* present on raw beef. These outbreaks ended after the required time and temperature of cooking were changed in 1980 and 1981.[136] New technologies may reduce contamination of poultry during slaughter and processing, including trisodium phosphate dips that rinse

Salmonella off carcasses, air-cooling devices to replace the cold water chill tanks, better eviscerators that decrease the likelihood of rupture of the crop or gut, and better decontamination procedures for the machinery used. Irradiating raw meat and poultry would be a singularly effective step in controlling salmonellosis, but one which thus far has not been a major commercial success.[137] Outbreaks traced to toasted oat breakfast cereal, peanut butter, and other low-moisture-processed foods illustrate the hazard that foods can be contaminated in the food factory environment, leading to widespread outbreaks.[138,57] Vigilance by the industry itself, and rigorous application of the HACCP approach to make sure that such foods are properly processed and do not become recontaminated could prevent such outbreaks.

Salmonella Enteritidis has been shown to contaminate the contents of the intact egg, as a result of ovarian infection in the hen. The hens themselves may be infected *in ovo* or by contact with infected mice or a contaminated environment. In response to the *S.* Enteritidis epidemic, many states adopted Egg Quality Assurance Programs, a series of control measures including screening flocks for infection, pasteurizing the eggs from infected flocks, better rodent control in henhouses, and a requirement for refrigeration of eggs during transport and distribution, so that a small number of contaminating *Salmonella* do not have the opportunity to multiply. These measures may explain the recent decline in *S.* Enteritidis infections in states where these measures were adopted, especially in the northeast and the mid-Atlantic region, where they were first pilot tested.[139] At the same time, hospitals, nursing homes, and restaurants are switching from shell eggs to pasteurized eggs and traditional recipes that use raw egg, such as homemade ice cream, eggnog, and Caesar salad, are being rewritten. Eggs that are pasteurized in the shell are now commercially available.

Licensing and inspection of food-service establishments, particularly when coupled with food-handler education and certification, is an integral part of the public health effort to prevent foodborne illnesses of all sorts, including salmonellosis. Educating consumers themselves is a constant effort, requiring the cooperation of the food production and retailing industries, the education establishment, and public health officials.

Many control measures are implemented to prevent person-to-person transmission and contamination of food by ill food workers. The potential for these types of transmission makes it prudent to exclude from work food handlers or healthcare workers with acute *Salmonella* enterocolitis, and convalescent persons in sensitive occupations who continue to shed the organism are often excluded until stool cultures no longer yield *Salmonella*. The risk posed by the entirely asymptomatic shedder is low and does not justify routine periodic stool cultures of food handlers or healthcare providers. Nosocomial salmonellosis can be controlled by taking routine precautions to prevent transmission of enteric organisms from patients with acute salmonellosis, including strict hand washing and other basic tenets of hospital infection control.[67]

Reptile-associated salmonellosis also has required specific control measures. The ban on the sale of small pet turtles in 1975, following unsuccessful efforts to educate the turtle-owning public in methods to reduce risk, led to a dramatic decrease in the isolation rate of turtle-associated serotypes.[23] However, outbreaks associated with small turtles continue to occur, possibly through exceptions to the federal prohibition.[140] It is unclear whether efforts to educate the public about the risk of iguana-associated salmonellosis will provide sufficient prevention.[89]

10. Unresolved Problems

There are many control points in the chain of transmission of *Salmonella*. However, it is difficult to achieve coordinated control efforts among the many industrial, professional, and regulatory groups involved. Salmonellosis will continue to be an important public health problem in the industrialized world and an increasing problem in developing countries. Although the pathogen has been well known for more than 100 years, fundamental questions remain unanswered. Microbiological issues in need of further study include clarifying the mechanisms of virulence, which may ultimately permit the successful development of vaccines. More rapid and sensitive means of detecting the organism in foods or the food production environment are needed if programs of food safety that depend on bacteriologic monitoring are to be widely implemented.

Several important clinical issues are unresolved. The risk factors for invasive infection in infants, particularly meningitis, are poorly defined and the use of antimicrobials to treat *Salmonella* enterocolitis in infants to prevent invasive infections is presently unproven. The paradoxic and important ability of the organism to persist in the individual who is treated with antimicrobials, even when the organism is sensitive to those antimicrobials in vitro, needs to be explained. Developing a treatment that would eliminate long-term carriage would be of benefit. Antimicrobial resistance of strains isolated from humans in the United States poses clinical challenges for the treatment of invasive infections and increases the risk that resistant salmonellosis will complicate the therapy of other infections. The problem of antimicrobial resistance would benefit greatly from a rational approach to

defining the choice and indications for use of antimicrobials in animal husbandry. Invasive infections are likely to be an increasing clinical problem, as the population ages and AIDS remain prevalent.

Unresolved public health issues include the need to incorporate population-based estimates of the burden of salmonellosis with information about the sources of these infections identified in outbreak investigations and sporadic case–control studies. In this way, the relative contributions of various foods, such as poultry products and produce items, and nonfood exposures, such as reptile pets, to the overall problem can be calculated. To take full advantage of opportunities provided by detection of outbreaks through surveillance enhanced by molecular subtyping, improved methods to rapidly investigate widely distributed outbreaks are needed. These outbreaks are challenging as they are often related to low-level contamination of a commercial food item. Expanded international collaboration can help identify and control foodborne sources of infection in our increasingly globalized food supply. Effective prevention strategies need to be demonstrated for salmonellosis in infants and HIV-infected individuals. Data obtained at slaughter could be used to target control efforts in the source flocks or herds. Better understanding is needed of the complex ecologies that may link animal reservoirs to plants that we eat. Reducing the contamination of our food supply will require a continuing effort to identify specific critical control points at the farm, the packing shed, the slaughterhouse and rendering plant, and the food-processing factory and to implement cost-effective control measures.

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CHAPTER 33

Shigellosis

Gerald T. Keusch

1. Introduction

Shigellosis is an acute, infectious, inflammatory enteritis of humans and, on occasion, subhuman primates. Because it is often clinically manifested by the dysentery syndrome (a triad of frequent small-volume bloody-mucoid stools, abdominal cramps, and tenesmus) it is often referred to as bacillary dysentery to distinguish this infection from amoebic dysentery. However, watery diarrhea without blood or mucus is common in infection due to all *Shigella* species and may be the only clinical manifestation of infection. In addition to these clinical presentations, there are a myriad of non-intestinal manifestations described in shigellosis, some common and some rare, that without doubt qualify the organism as a protean pathogen. The objective of this chapter is to present the salient features of shigellosis in sufficient detail to understand the spectrum of the disease and its distinctive epidemiology.

2. Historical Background

Dysentery has long been clinically recognized. The classic syndrome is depicted in the Old Testament, and it is mentioned in the descriptions of war, so common in our history, from Thucydides in the Levant to Montgomery at the Battle of El Alamein and more recently in the Persian Gulf and Iraq wars.

There are two principal causes of the dysentery syndrome – *Shigella* species and *Entamoeba histolytica* – and a number of minor causes, including *Salmonella* and *Campylobacter*. Under circumstances of epidemic dysentery, *Shigella* is more likely to be the agent involved, while sporadic disease in adults associated with liver involvement is probably due to *E. histolytica*. However, in individual patients, the specific etiology of the syndrome can be determined only by laboratory investigation. This first became possible after Losch described *E. histolytica* and demonstrated its virulence in dogs in 1875, and Councilman and Lafleur reported the anatomic criteria for the diagnosis in humans in 1891. However, the problem of assigning etiology to cases of dysentery in which neither the organism nor characteristic histology was present remained. The loop was closed in 1898 by Dr. Kioshi Shiga,[1] who isolated the organism now known as *Shigella dysenteriae* 1 (Shiga's bacillus) from the stools of patients with epidemic dysentery in Japan during a particularly widespread (almost 90,000 cases) and severe (25% mortality) outbreak at the end of the 19th century. Shiga also confirmed the relevance of this organism by demonstrating the presence of specific serum agglutinins in individuals convalescent from the disease but not in the healthy or in patients with other diseases. In fact, the identical organism had been reported a decade earlier by Chantemesse and Widal as the cause of nonamoebic dysentery, but the description was less complete and lacked the epidemiological proof of Shiga's study, and so it is the Japanese microbiologist who is honored in the genus name. Two years later, Shiga's results were confirmed by Flexner in the Philippines and by Kruse in Germany.

Soon thereafter, Kruse isolated a similar but serologically distinct organism during an outbreak of "asylum dysentery," an epidemic disease common in institutions for the mentally ill, which he called *Bacillus pseudodysentery*. Identical organisms were soon described by Flexner, Strong, Musgrave, and Castellani, who suggested the name *B. paradysenteriae* instead. Todd referred to this same strain as *B. dysenteriae* Flexner, and to clear the confusion the name *S. flexneri* was ultimately adopted. A third species was defined by Duval, Castellani, and Kruse,

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who found a serologically distinct, late lactose-fermenting *Shigella*, which Kruse originally called *B. pseudodysenteriae* type E. To honor the subsequent extensive work of Sonne on Kruse's type E organism, the name was first modified to *B. dysenteriae* Sonne and then shortened to *S. sonnei*.

The final stage in classification of shigellae began in 1929 with Boyd's studies in Bangalore, India, of non-lactose-fermenting, mannitol-positive organisms resembling *S. flexneri* that were not agglutinated by *flexneri* antisera. Boyd showed that there were distinctive serological reactions with homologous antisera to the inagglutinable Flexner group, and on this basis such strains were classified together as *S. boydii*. By 1938, then, fully 40 years after the first complete description of the prototypic organism, *S. dysenteriae* 1, the four species of *Shigella* were defined (*dysenteriae* [10 serotypes], *flexneri* [6 serotypes], boydii [15 serotypes], and *sonnei* [1 serotype but multiple colicin types]).

At the beginning of the 20th century it was commonly believed that pathogenic bacteria exerted their effects via toxins, following the example of tetanus and diphtheria. Investigators therefore looked for and found that cell-free culture supernates of Shiga's bacillus, but not the other species, were toxic to animals, causing a characteristic limb paralysis followed by death in certain species.[2] This toxic activity was therefore called Shiga neurotoxin. Over the next 50 years, the neurotoxic material was clearly shown to be a protein distinct from lipopolysaccharide and, when partially purified, to be one of the most potent known poisons. From the time of its discovery, however, neurotoxin remained a biological activity in search of pathogenetic relevance until 1972 when neurotoxin-containing cell-free preparations from recent epidemic isolates of Shiga's bacillus were shown to be enterotoxic as well, capable of inducing both fluid secretion by rabbit small intestine and an inflammatory enteritis resembling the colitis of human dysentery.[3] Although this finding suggested the importance of the toxin in pathogenesis of the intestinal phase of shigellosis, the chromosomal gene for Shiga toxin (Stx) is restricted to *S. dysenteriae* 1 and it is considered to be a salient accessory factor involved in pathogenesis of the particularly severe disease associated with this species. However, this may change as the closely related Stx-1 gene present in a naturally transferable temperate phage has been identified in *S. dysenteriae* 4 causing human illness.[4] Other enterotoxic proteins have been identified in non-dysenteriae 1 strains isolated from diarrhea patients, indicating a potential role in pathogenesis.[5] Certain serotypes of *Escherichia coli*, for example O157:H7, produce toxins closely related to Shiga toxin and play a role in pathogenesis of hemorrhagic colitis and hemolytic–uremic syndrome.[6]

3. Methodology

3.1. Sources of Mortality and Morbidity Data

It is difficult to accurately determine morbidity or mortality rates due to shigellosis, except during epidemics or in microbiologically proven hospital series. There is first of all a "numerator problem" to identify shigella infections because in the absence of bloody diarrhea or frank dysentery the clinical manifestations of shigellosis are indistinguishable from other bacterial or viral diarrheas. Without laboratory confirmation the etiology of the illness cannot be determined, unless the case is part of a cluster of clinically related illnesses, as in a common-source or a day-care center outbreak, at least one of which is laboratory confirmed. The laboratory diagnosis of an individual case currently depends on good microbiological technique. Unfortunately certain media commonly used for isolation of enteric pathogens are inhibitory to the growth of shigellae. In addition, prior treatment with antibiotics may prevent growth of the pathogen and render cultures unreliable. Good laboratory practice and the use of multiple media are essential to maximize yields. Cultures taken in the field are often transported to a laboratory for processing using a transport medium. However, shigellae do not survive well in certain commonly used transport media, such as Cary–Blair, resulting in underreporting. Second, there is also a "denominator problem," to identify patients at risk of shigella infection. Not only are some age groups less susceptible, but also asymptomatic and mild infections may occur more frequently than previously suspected.[7] *Shigella* infection is most often identified in patients, usually children, who either have the dysenteric or bloody diarrhea forms of the illness or complications, such as seizures. More mildly affected patients, particularly those without grossly bloody stools, are usually treated symptomatically and specific etiology is not determined. The problem of underdiagnosis is even more pronounced in many developing countries, where access to health care facilities is limited and laboratory services are, as a general rule, not available.

Available data generally come from three sources: (1) hospital-based reports, (2) etiologic investigations of diarrhea in the community setting, and (3) national surveillance. The first source will miss most of the disease in the community. The third, such as the national surveillance system maintained by the US Centers for Disease Control and Prevention (CDC), depends on multiple reporting sources based on cultures submitted to local and state health departments. This is now supplemented by an active surveillance for foodborne pathogens known as FoodNet, carried out in 10 states in the US with a catchment area of some 44 million people.[8]

Community studies of the second sort provide the most reliable estimates of morbidity or mortality; however, these data are usually time and place specific and are not necessarily applicable universally.

3.2. Surveys

Both bacteriologic and serological methods have been used in prospective community-based surveys of the incidence and prevalence of *Shigella* infection. However, bacteriologic surveys can underestimate the true infection prevalence because the stool sample may be inadequate or poorly handled so that organisms die before they can be cultured on appropriate media or because patients are sampled either too late in the course of their illness or after antibiotic therapy has been initiated.

A variety of serological methods have been employed for detecting both acute infection as well as evidence of past infection, but their usefulness is limited because there can be serological cross-reactivity between *Shigella* and other genera of bacteria as well as between different serotypes of *Shigella*. In addition, titers may wane rather quickly after infection. Serotype-specific enzyme-linked immunosorbent assays (ELISAs) have been incorporated into test kits which have been validated in the field and appear to be sensitive and specific.[9] A new dipstick version has recently been described for *S. flexneri* 2a.[10] Multiplex PCR assays for shared *Shigella* virulence traits, such as invasion plasmid antigens or other outer-membrane proteins or toxins, have been developed, but their utility for diagnostic or epidemiological purposes has not been fully explored.[11]

3.3. Laboratory Diagnosis, Isolation, and Identification of the Organism

Shigellae are slender, gram-negative, nonmotile rods. They are members of the family Enterobacteriaceae and tribe Escherichia and are closed related to *E. coli*. Routine isolation and identification of *Shigella* species involve the use of selective bacteriologic media that spotlight certain biochemical properties of the genus and allow a presumptive microbiological diagnosis. Identity can be quickly confirmed by the use of serological agglutination reactions, employing group-specific anti-O (somatic) antigen sera. Unfortunately, many of the commercially available typing sera are of poor quality, being too dilute or too cross-reactive to identify group- or type-specific antigens.[12] Even so, the grouping sera are reasonably good for *S. dysenteriae* 1, *S. flexneri* (except *flexneri* 6), and *S. sonnei* bearing form I antigen, which are the most important isolates to identify in the United States.

Feces is the most frequently submitted specimen for isolation of *Shigella*. Feces is a complex ecosystem of many different organisms, therefore laboratory isolation uses special media with two purposes in mind: (1) to suppress the growth of the usual nonpathogenic normal flora that might numerically overwhelm and obscure the growth of *Shigella* and (2) to dramatically contrast the colonial appearance of the possible pathogen with that of the common commensal so that suspect colonies may be picked for further study. This is accomplished by inclusion in the media of substances inhibitory to the growth of nonpathogens and a dye indicator system to detect rapid fermentation of lactose, since all shigellae are unable to perform this reaction, while most commensals can. A variety of media have been developed for this purpose; the most successful and currently used are MacConkey's bile salt, xylose–lysine–deoxycholate (XLD), Hektoen enteric (HE), and tergitol-7-triphenyl tetrazolium chloride agars. These media permit the growth of all *Shigella* species reasonably well, are inhibitory to normal flora, and clearly distinguish the lactose-negative organisms by color reactions. *Salmonella–Shigella* (SS) agar is far too inhibitory to shigellae, especially to *S. dysenteriae* 1, to be recommended for general use, particularly if only one differential medium is used.[13] Indeed, use of several media is highly recommended to maximize the yield of positives. When the number of organisms in the sample is small, isolation may be facilitated by brief enrichment in broth inhibitory to nonpathogens, such as Hajna's gram-negative broth. After 6–8 h of incubation of the sample in broth, the resulting growth is streaked onto the same differential media used in direct isolation.

Three technical details should be followed to minimize the number of false-negative cultures: (1) whenever possible, inoculate from a stool sample, rather than a swab, selecting blood-tinged mucus plugs as the source of culture; (2) if a swab is to be used, be certain that it passes beyond the anal sphincter; and (3) if there is to be any appreciable delay in processing the sample, place it in buffered glycerol–saline-holding medium.[14] Some evidence suggests that extending incubation of plates to 48 h can increase the yield of positives by almost 5% with no additional cost and minimal additional technician time.[15] In endemic regions of the world this may be a useful approach to specific diagnosis.

Lactose-negative colonies are picked to a medium such as triple sugar iron (TSI) agar, which allows confirmation of the lactose-negative phenotype and detects glucose fermentation and lack of motility, which are additional properties of shigellae. Both the agar surface (slant) and the butt are inoculated with the colony to be tested, to achieve aerobic and anaerobic conditions, respectively. In the butt, fermentation of glucose results in a pH change and a change in the

color of the phenol red indicator to yellow (acid) but without gas bubbles, while on the slant, the failure of the organism to use lactose (or sucrose, which is also contained in TSI) precludes sufficient drop in pH to change the pink (alkaline) color. TSI also distinguishes the non-lactose-fermenting enteric pathogens in the genus *Salmonella*, for which, in contrast to *Shigella*, a black reaction product is deposited due to H_2S formation. The presumptive diagnosis is then confirmed by serological agglutinations using group-specific antisera. More thorough biochemical studies can be done, but this is rarely necessary for clinical needs, and the criteria enumerated above, included in standard schemata for the identification of Enterobacteriaceae, are sufficient for diagnostic purposes.

4. Biological Characteristics of the Organism

The ability to invade epithelial cells has been considered the single most important characteristic of *Shigella* mediating its characteristic pathogenesis. Studies over the past decade have shown that invasiveness per se is just one characteristic of a set of traits that allows the organism to enter the host cell, then multiply, spread within the infected cell and directly from cell to cell, and most importantly for virulence to activate a number of cell-signalling and cytokine pathways. Ultimately these events kill the contiguously infected cells and result in mucosal ulceration.[16] The cell biology of *Shigella* is a highly complex and exquisitely interactive process between microbe and host, and in contrast to previous interpretations, cell death is the response to and not the cause of inflammation. These microbial properties are enlisted in a sequential manner during infection and are dependent on multiple virulence genes encoded on both the chromosome and a large 120- to 140-MDa plasmid present in all virulent *Shigella* and the phenotypically similar enteroinvasive *E. coli* (EIEC).[17] These virulence genes are, in turn, under the control of multiple regulator genes.[18] Once invasion of a single host cell has occurred, the entire process from invasion by a process analogous to phagocytosis, escape from the phagocytic vesicle, intracellular multiplication, cell-to-cell spread, and death of the newly infected cell can occur without exposure of the organism to the external milieu. In this manner, *Shigella* secures a secure ecological niche for itself, sequestered away from host antibacterial immunity. This protected niche is one of the factors permitting establishment of infection in a significant number of adults challenged with as few as 100–200 viable *Shigella*[19] (Table 1). The tiny infective dose, in turn, permits effective contact spread from host to host without the need for enrichment growth in some vehicle such as food or water.

Table 1. Infectious Inoculum in Experimental Shigellosis in Normal Adult Volunteers[a]

Species (strain)	Dose[b]	Number of volunteers	Number (%) ill
S. dysenteriae 1			
(A1)	200	8	3 (38)
	10^4	6	2 (33)
(M131)	10	10	1 (10)
	200	4	2 (50)
	2×10^3	10	7 (70)
	10^4	6	5 (83)
S. flexneri 2a			
(2457-T)	100	36	14 (39)
	180	36	9 (25)
	5×10^3	49	28 (57)
	10^4	103	58 (56)
	$\geq 10^5$	59	38 (64)
S. sonnei			
(53 G)	500	58	26 (45)

[a] Data from DuPont et al.[13]
[b] Estimated number of bacteria given in oral inoculum, suspended in 30 ml of milk.

4.1. Invasiveness and Intercellular Spread

It is now clear that invasion of host cells by virulent *Shigella* occurs within a host cell membrane-bounded vesicle by "non-professional" phagocytic cells.[16] Invasion must be followed by escape from the vesicle to the cytoplasm where multiplication of the organism is possible, spread within each host cell, and invasion of adjacent cells, since interruption of any of these stages renders the organism avirulent. The process has been arbitrarily divided into four stages, including (1) entry into the host cell, (2) vesicle lysis and bacterial growth, (3) intra- and intercellular spread, and (4) death of the host cell. Distinctive genes are required for each step, and while this model for virulence has been developed largely on the basis of studies of *S. flexneri* strains, the more limited number of studies with other *Shigella* serotypes and the biologically similar enteroinvasive *E. coli* (EIEC) indicate the same mechanisms are involved.

4.1.1. Invasion. Earlier studies of cell invasion were carried out in non-intestinal tissue culture models and demonstrated that *Shigella* invade directly across the apical membrane of the host epithelial cells. When more relevant models are used it is clear that the initial site of invasion from the intestinal lumen is probably the antigen-sampling M cell rather than the enterocyte.[20] Inflammatory reactions in the lamina propria result in an inpouring of neutrophils which migrate between intestinal epithelial cells to the lumen. This opens up the intracellular tight junctions which, in turn, allow large numbers of *Shigella* to reach the basolateral membrane of the enterocyte which can be directly breached by the organism, markedly accelerating the process.

Organisms enter the cell within a vesicle formed from host cell membrane, initiated by at least three surface proteins of the microbe called invasion plasmid antigens (IpaB, C, and D). These proteins are encoded by a plasmid operon common to all invasive and virulent shigellae and EIEC and are transported to and inserted in the outer membrane of the organism under the control of two regulatory gene sets, *mxi* (membrane export of Ipa) and *spa* (surface presentation of invasion plasmid antigens). Ipa proteins lead to complex changes in the cytoskeleton of the host cell that underlie vesicle formation and bacterial entry.

4.1.2. Vesicle Lysis and Intracellular Multiplication. To be virulent, *Shigella* must also rapidly escape the vesicle to reach the cytoplasm of the host cell. Lysis of the vesicle has been associated with a temperature- and pH-dependent contact membrane lytic activity, sharply increased at the acid pH achieved within phagocytic vesicles.[21] The molecular basis for vesicle lysis appears to reside in the Ipa proteins involved in bacterial invasion since nonpolar inactivation mutants of IpaB, C, or D are inhibited in both entry and lysis of the vesicle. Intracytoplasmic multiplication of *Shigella* begins soon after vesicle lysis and if inhibited by a variety of methods the organism loses virulence.

4.1.3. Intra- and Intercellular Spread. Although *Shigellae* do not have flagella and are therefore nonmotile, they must move within the host cell cytoplasm to reach the plasma membrane to be in a position to invade adjacent cells from their intracellular niche. An elaborate mechanism allows the organisms to use the host cell cytoskeleton to accomplish this, via the plasmid-encoded protein IcsA.[22,23] IcsA is expressed during bacterial division at the back pole of the organism, defined in terms of its subsequent direction of movement. IcsA initiates localized actin polymerization, and interactions with other host cell proteins, such as plastin, result in actin cross-linking and contraction, which provide a forward propulsive force. This so-called actin motor is energized by ATP also generated by IcsA, which is an ATPase that is regulated by cyclic nucleotide-dependent host cell protein kinases that phosphorylate IcsA.[24] Bacteria defective for phosphorylation demonstrate enhanced microbial spread, whereas protein kinase inhibitors block host cell invasion. The process of cell-to-cell invasion is initiated when *Shigella* reach the host cell membrane where they create long finger-like protrusions, still membrane bounded, into the adjacent cell. These subsequently fuse with the plasma membrane of the second cell allowing the organism, now within a double membrane vesicle, to reach the cytoplasm of the newly invaded cell. Lysis of this vesicle again places the bacterium in the cytoplasm of the newly invaded cell.

4.1.4. Cell Death. The end result of invasion and cell-to-cell spread is host cell death, which results in the characteristic ulcerations of bacillary dysentery, but whether or not epithelial cell death is important for pathogenesis remains uncertain. There is good evidence that invasion of macrophages in the lamina propria by *Shigella* results in cell death by a process of apoptosis, [25] and this leads to the release of cytokines and other mediators that play a role in orchestrating the inflammatory response. IpaB is essential for this phase of pathogenesis, since strains with mutational inactivation of IpaC or D but still expressing low levels of an inserted *E. coli* hemolysin to allow escape from the phagocytic vesicle are able to induce apoptosis whereas an IpaB mutant does not.[26]

4.2. Acid Resistance

Impaired gastric acid secretion, from whatever cause, increases the risk for *Shigella* infection. This is because gastric acid is an effective barrier for acid-sensitive organisms, reducing the number of viable organisms entering the more hospitable small intestine. The ability to resist acid pH is a non-specific but efficient accessory virulence trait. Acid resistance helps to explain one of the unique characteristics of *Shigella* pathogenesis, the ability to cause infection and illness with a very small inoculum.[27] This is so important to *Shigella* lifestyle that more than one mechanism has been identified, including a glutamate-dependent mechanism regulated by a stationary growth phase sigma factor encoded by *rpoS* and a glutamate-independent mechanism controlled by the iron-regulated *fur* gene.[28]

The acid resistance phenotype is growth phase dependent, expressed when the organisms reach late log and stationary phase in an acid pH environment, conditions present in the large bowl.[29] This is ideal, as the organisms become acid resistant as they enter the environment via the feces and thus are already programmed to survive stomach acid when ingested by a new susceptible host. There is a trade-off, however, at least in vitro, as acid-resistant bacteria have a reduced capacity to invade mammalian cells. Acid resistance is lost as the organisms encounter neutral to alkaline pH, as they do in the small bowel, which restores their ability to rapidly multiply.

4.3. Other Virulence Determinants

Soluble proteins, such as iron-binding siderophores, provide an advantage to *Shigella.* Iron uptake is essential for bacterial growth, and such proteins are required to bind and transport iron into the bacterial cell. While there appears to be no difference in growth or invasive capacity between

siderophore-positive or -negative organisms in tissue culture, this is an environment in which iron is provided and is readily available. In contrast, in vivo free iron is virtually completely protein bound and functionally unavailable, and the kinetics of infection with siderophore mutants is delayed, indicating the importance of iron uptake mediated by siderophores.[30]

Some evidence suggests that superoxide dismutase (SodB) produced by *Shigella* promotes microbial survival and so enhances virulence by blocking the bactericidal effects of host-derived reactive oxygen radicals.[31] A *sodB S. flexneri* mutant is extremely sensitive to oxidative stress and is readily killed by macrophages and neutrophils and has been shown to be attenuated in certain animal models of virulence.

The specific structure of the O side chain of the somatic antigen of lipopolysaccharide (LPS) endotoxin may also affect virulence in some *Shigella* strains. Conjugal transfer of *E. coli* somatic antigen 8 into *S. flexneri* 2a renders the organism avirulent.[32] However, if *E. coli* O-25 antigen is used instead in identical experiments there is no change in virulence. The latter has been shown to resemble *S. flexneri* 2a somatic antigen in its high mannose content. Rough *Shigella* lacking O side chains invade and multiply intracellularly but do not spread from cell to cell, and they too are avirulent in vivo. Moreover, they do not cause disease in humans accidently infected in the laboratory, in contrast to smooth (O-antigen complete) strains.[33] Lack of virulence of rough mutants appears to be, in part, the failure to transport and correctly localize IcsA in the bacterial cell outer membrane.[34] Mutations in the LPS core region biosynthetic pathway delay cell-to-cell spread in vitro as well as reduce the extent of the inflammatory mucosal reaction in vivo.

4.4. Toxins

Shiga toxin (Stx), a cytotoxic protein toxin made by *S. dysenteriae* 1, is lethal when injected into certain species of mammals and is of the same order of potency as botulinum

Table 2. Selected Recent Surveys of Antimicrobial Resistance of *Shigella* Species

				Percent (%) susceptible to the listed agent[a]					
Year	Location	*Shigella* spp.	Number	Amp	TMP-SMX	Nal	Pivam	Cipro	Ceph
1999–2002	United States	*dysenteriae* 1	7	29	43	100	–	100	100
		flexneri	295	24	60	98	–	99	100
		boydii	18	100	61	28	–	100	100
		sonnei	1,278	21	52	99	–	100	100
2001–2002	Bangladesh	*dysenteriae* 1	35	12	10	2	91	98[d]	100
		flexneri	136	22	32	82	99	97[d]	100
		boydii	52	61	58	67	100	83[d]	97
		sonnei	43	84	13	46	100	70[d]	95
1994	Bangladesh	*dysenteriae* 1	229	2	0.5	2	95	100	100
		flexneri	461	44	33	95	95	100	100
		boydii	175	74	51	99	99	100	100
		sonnei	72	86	32	77	97	100	100
1991–1992	Israel	*flexneri*	39	49	60	100	NT	100	80
		sonnei	557	16	14	100	NT	100	99
1993	Spain	*flexneri*[b]	14	36	50	NT	NT	100	NT
		flexneri[c]	35	40	63	NT	NT	100	NT
		sonnei[b]	26	69	92	NT	NT	100	NT
		sonnei[c]	22	68	46	NT	NT	100	NT
1989–1992	Brazil	*flexneri*	21	6	19	10	NT	100	93
1991	Guatemala	*flexneri*	36	NT	25	NT	83	NT	NT
		sonnei	15	NT	13	NT	93	NT	NT
1990	Burundi	*dysenteriae* 1	50	0	0	0	100	100	100
		flexneri	23	87	87	100	100	100	100
1990	Canada	*dysenteriae* 1	24	54	71	NT	NT	NT	NT
		flexneri	254	33	79	NT	NT	NT	NT
		boydii	30	43	73	NT	NT	NT	NT
		sonnei	290	61	62	NT	NT	NT	NT

[a] Antibiotics: Amp, ampicillin; TMP-SMX, trimethoprim–sulfamethoxazole; Nal, nalidixic acid; Cipro, ciprofloxacin; Ceph, third-generation cephalosporin.
[b] Local isolates.
[c] Traveler's diarrhea isolates, primarily from developing countries.
[d] Reflects intermediate resistance.

and tetanus toxins.[35] Stx is not an essential virulence factor, for just one species, *S. dysenteriae* type 1, produces the toxin. However, Stx-positive wild-type *S. dysenteriae* cause a more severe illness than toxin-negative strains in both rhesus[36] and human volunteers.[37] Shiga toxin-producing *Shigella* and *E. coli* can result in hemolytic–uremic syndrome and thrombotic thrombocytopenic purpura, whereas toxin-negative strains do not.[38] These three toxins, Stx and *E. coli* Stx-1 and Stx-2, represent a closely related family of heterodimeric proteins composed of one enzymatically active A subunit and a cluster of five identical B subunit monomers that mediate binding of toxin to target cell receptors. All share the same enzymatic specificity and inactivate 28S rRNA of the mammalian ribosome and block protein synthesis.[39] In rabbit ileum Stx inhibits the synthesis of brush border enzymes, including lactase, sucrase, and alkaline phosphatase, and results in blunted villi with increased numbers of inflammatory cells in the lamina propria and extrusion of epithelial cells into the lumen.[40] The B subunit pentamer for all of these toxins binds to the same receptor, a glycolipid terminating in a galactose-α1→4-galactose disaccharide.[41]

Two other protein toxins designated *Shigella* enterotoxin (ShET)-1 and -2 are present in some strains, the former exclusively in *S. flexneri* 2a.[42] These toxins alter electrolyte transport and cause net fluid secretions in animal models. Neutralizing antibody is present in humans, indicating that they are produced in vivo. Deletion of the genes for the ShET toxins in a potential vaccine strain of *S. flexneri* 2a further attenuates the organism in human volunteers, suggesting a clinical role for these proteins.[43]

4.5. Antimicrobial Resistance

Resistance to antimicrobial agents commonly used in the treatment of shigellosis is a major problem in developed and developing countries. Increasing resistance to ampicillin and trimethoprim–sulfamethoxazole (T-SMX), the least cost drugs of choice for the treatment of drug-susceptible shigellosis, has rendered these drugs virtually useless.[44] Nalidixic acid, a first-generation quinolone, was initially effective and used in developing countries for drug-resistant *S. dysenteriae* type 1 and *S. flexneri* infection but is now ineffective because of acquired resistance. The second-generation fluoroquinolone ciprofloxacin has become the drug of choice, but until generic versions became available was an expensive option for many developing countries. However, strains with decreased susceptibility are beginning to appear.[45] Ceftriaxone and azithromycin are alternative, albeit expensive, choices, but unfortunately resistance is also emerging (Table 2). Part of the problem of emerging resistance is the frequency with which antibiotics are inappropriately used.[46]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

In 1986, it was estimated that 140 million cases and 576,000 deaths occur annually due to *Shigella* infection in children under 5 years of age, even excluding data from China.[47] In 1999, Kotloff et al. conducted a comprehensive review and estimated that there were more than 113 million episodes yearly in children under 5 years in developing countries, or 0.2 episodes of bloody diarrhea per child per year, with as many as 600,000 deaths.[48] These estimates are problematic because routine surveillance does not usually record bloody diarrhea separately from watery diarrhea, nor are culture techniques often employed. This may have the effect of reducing the perceived incidence of shigellosis, especially because grossly bloody diarrhea is just one of the manifestations of infection. It is not surprising, then, that a recent thorough and prospective culture-based surveillance project in six Asian countries documented a higher incidence of *Shigella* infection than previously believed, with an incidence of 1,320/100,000 in children under 5 years.[49] Overall, a *Shigella* species was isolated from 5% of diarrheal stool samples, an incidence of 210/100,000 in subjects of all ages. These data, well documented and collected, revealed that two thirds of the isolates were *S. flexneri*, except in Thailand where *S. sonnei* accounted for 85% of the strains recovered. In addition, one third of a sample of culture-negative stools was positive for the IpaH antigen of *Shigella*, suggesting even higher rates of infection than identified by culture methods, most likely due to prior antibiotic use and/or suboptimal microbiological technique. Shigellosis continues to be a major problem in the developing world, especially in children under 5 years of age.[50] Given the continuing drop in estimated diarrheal mortality to 1.5–2.5 million per year during the 1990 s, associated primarily with the increasingly widespread use of oral rehydration therapy (ORT), and the limited impact of ORT on *Shigella* lethality, a substantial portion of the deaths remaining after subtraction of the 600,000 deaths estimated to be due to rotavirus are probably due to *Shigella.*[51] Progress in the control of shigellosis in the developing world has, as a result, lagged behind in the control of watery diarrhea.

In the United States, CDC estimates that there are still some 450,000 persons infected with *Shigella* spp. annually, with over 6,000 hospitalizations.[52] Endemic isolation rates for all *Shigella* spp. derived from the CDC's

national laboratory-based surveillance system has approximated around 6 per 100,000 between 1967 and 1988, although there were periods of increased endemicity, when rates increased to 9–10 per 100,000.[53] Rates since then have dropped slightly, with an annual incidence of 5.6 cases per 100,000. There continues to be year-to-year variation, with a peak of 7.6/100,000 in 1993 and a low of 3.7/100,000 in 1999. In addition, case rates are not uniform across the United States, ranging from 10 cases per 100,000 in the Western states to 3.9 cases per 100,000 in the Northeastern states. The downward trend in the United States was more apparent during the years 1998–2002, when incidence dropped to 3–4/100,000. These trends are confirmed by data generated from the CDC's Foodborne Diseases Active Surveillance Network, FoodNet. Compared to the baseline period, 1996–1998, the isolation of *Shigella* decreased by 35% in 2006.[54] Based on proven isolations, the proportion of all samples positive for *S. sonnei* increased from 64% in 1989 to almost 84% in 2002. When separated by species, the overall reduction in shigellosis during the surveillance period has been primarily due to a decrease of 64–83% in the frequency of non-*sonnei Shigella* species, particularly *S. flexneri*. Compared with 1990, *Shigella* isolation rates at the millennium were lower in urban, semiurban, rural, and low-income counties, and most dramatically in counties with a relatively high proportion of Native American or African-American residents. As in the past, the overall isolation rate in children under 5 years (20.6/100,000) was five times greater than in adults over 20 years of age (<4/100,000).

There are several population groups in the United States at greater risk of shigellosis than the general population. These include Native Americans, who, in addition, are more commonly infected with *S. flexneri* than the rest of the American population. The developmentally disabled and mentally retarded are also at increased risk because of the difficulty in controlling fecal contamination in their environment. This problem has historically been referred to as "asylum dysentery." Just a few decades ago, as many as 10% of long-time residents of residential schools were infected with *Shigella* at any time, and new admissions had an amazing incidence of 37,000 per 100,000. Long-term, intermittent excretion of the organism was present in 7% of residents, due either to prolonged carriage or frequent reinfection. In New York state from 1982 to 1987, the relative risk (RR) for diarrhea outbreaks at summer camps for the developmentally disabled compared to summer camps for normal children was 8.6, and all the *Shigella* infections occurred in the former (RR = infinity).[55] Now that institutionalization is no longer the norm, other situations in which children are clustered together, such as summer camps, represent the greatest challenge. Finally, and for the same reasons, all children in day-care centers have a higher incidence of shigellosis.[56] With the increase in the number of children in day-care facilities in recent years, these centers are playing an increasingly important role in transmission of the disease in the United States. In prospective studies in some centers, the incidence of culture-confirmed shigellosis has approached 7,000 per 100,000, with secondary cases in as many as one quarter of families when a child in day care developed shigellosis. In other settings, infection was first introduced into the household by a child attending day care in 25% of affected families investigated. Of concern, multidrug-resistant *S. sonnei* is increasingly being isolated in day-care outbreaks.[57]

The prototypic *Shigella* species, *S. dysenteriae* 1, was the cause of numerous outbreaks of shigellosis throughout the world in the first two decades of the 20th century, with high morbidity and mortality.[58] Inexplicably, the prevalence of this organism dropped sharply after 1920, and various serotypes of *S. flexneri* became dominant. After World War II, *S. sonnei* gradually replaced *S. flexneri* in industrialized nations,[59] while *S. flexneri* persisted in developing countries.[60] In the first decade of surveillance by the CDC in the United States from the mid-1960 s to the mid-1970 s, the proportion of *S. flexneri* went from 60.6% in 1964 to 15.5% in 1973, while the percent isolation of *S. sonnei* rose from 38.1 to 83.6%.[61] These trends have continued to the present. From 1970 through 1994, 269,890 confirmed isolates of *Shigella* were reported to the CDC through the national surveillance system.[62] Species information was available in nearly 98% (Table 3). Isolation of *S. sonnei* exceeded that of *S. flexneri* by 3:1, and the two species accounted for over 97% of all isolates. From 1989 to 2002, there were 208,368 laboratory-confirmed Shigella infections, and *S. sonnei* accounted for 71.7%, while 18.4% were *S. flexneri*.[52]

A similar change in prevalence has been observed in Israel, where *S. sonnei* overtook *S. flexneri* as the main isolate in the 1960s.[63] Between 1986 and 1991, the proportion of *S. sonnei* increased further from 60 to 91%, while

Table 3. Prevalence of *Shigella* Species in the United States, 1970–1994[a]

	Percent (%) of identified isolates during reporting period			
	1970–1979	1980–1989	1990–1994	2003
S. dysenteriae	0.6	0.8	0.5	04
S. flexneri	22.6	23.7	15.6	14.4
S. boydii	1.0	1.9	1.1	1.1
S. sonnei	75.8	73.6	82.8	80.2

[a] Adapted from the Laboratory-Confirmed *Shigella* Surveillance Annual Summary, Foodborne and Diarrheal Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA.

Table 4. Median Age of Persons Infected with *S. flexneri* or *S. sonnei*, United States, 1977–1991[a]

	Surveillance year, median age in years														
Serogroup	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
S. flexneri	9	6	10	11	11	19	20	23	21	21	22	22	18	18	18
S. sonnei	6	7	6	6	7	7	7	7	8	7	7	7	7	8	7

[a]Adapted from the Laboratory-Confirmed *Shigella* Surveillance Annual Summary, 1991–1992, Foodborne and Diarrheal Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA.

S. flexneri decreased from 29 to 8% over the same period.[64] In regions of Israel where both westernized and nonwesternized populations live in the same geographic area, *S. sonnei* was the dominant organism among the former (73.2%), whereas *S. flexneri* continued to predominate in the latter group (66.7%).[65] In Northern Israel during the same time frame *S. sonnei* was isolated more than four times more often (74%) than *S. flexneri* (21%) in hospitalized patients, whereas the relative frequencies in non-hospitalized patients was 87 and 10%, respectively.[66] Furthermore, *S. sonnei* was recovered in 82% of hospitalized Jewish patients with a positive culture but only 60% of hospitalized Arab children, who were far more likely to be poor and live in overcrowded settings. *S. flexneri* was particularly frequent among hospitalized infants, and was associated with Arab origin, large families, and residence in agricultural settlements.

5.2. Epidemic Behavior

Engrafted on this still unexplained, changing world picture in prevalence of the four *Shigella* species has been the reappearance of worldwide epidemic of *S. dysenteriae* 1 dysentery since 1969. After many years of playing a minor role in the etiology of shigellosis in Central America, *S. dysenteriae* 1 suddenly and dramatically became the dominant enteric pathogen in 1969–1970.[67] It resulted in epidemic spread of infection, affecting adults as well as children, with the index case often an adult, and it caused a degree of severity in the disease much beyond the accustomed level. For example, in 18 Guatemalan communities surveyed, the dysentery mortality increased from 39 per 100,000 in 1968 to 170 per 100,000 in 1969 when the outbreak occurred. All age groups were affected, the youngest and the oldest most significantly. The death rate for the entire country was estimated to be 250 per 100,000, varying from 334 per 100,000 in the lowlands, where there was more crowding, to 190 per 100,000 in the highlands. The case fatality rate was also quite high in untreated patients, ranging from 8.4% of community cases to 10–15% of those hospitalized because of acute illness. This rate decreased later in the epidemic when the cause was appreciated and appropriate therapy was employed. These features are in contrast to endemic shigellosis, principally due to *S. flexneri*, and to a lesser extent *S. sonnei*, primarily affecting young children in the under 5-year-old group, with the index case in families generally a preschool or school-age child and with mortality in adults an unusual event.

Epidemic *S. dysenteriae* 1 dysentery subsequently appeared in South Asia in the mid-1970 s and in Africa in the early 1980 s. After the initial large-scale outbreaks, it has become a part of endemic dysentery, with periodic surges in incidence. For example, prospective bacteriological data from 1978 to 1987 obtained in rural Bangladesh show a dominance of *S. flexneri* between 1978 and 1982 (56–67%), a rise in *S. dysenteriae* 1 prevalence between 1983 and 1985 (45–50%), and a return of *S. flexneri* in 1986 and 1987 (55–61%).[60] Continued surveillance for *S. dysenteriae* has also revealed a change in the prevalent types, from *S. dysenteriae* type 1, the dominant serotype in 1999 (76.4%), which declined to 6.5% in 2002, while *S. dysenteriae* types 2–12 were isolated with increasing frequencies, with types 2 and 4 accounting for nearly half of the total isolates.[55]

However, wherever *S. dysenteriae* type 1 lurks in the background, the potential for epidemic spread remains a threat. When a sudden influx of nearly three quarters of a million refugees fleeing Rwanda entered into Zaire in 1994, explosive sequential outbreaks of cholera and then *S. dysenteriae* 1 occurred, with a crude mortality rate of 200–350/100,000 per day and around 50,000 cumulative deaths.[68] This was followed by widespread acute malnutrition, especially in those with a recent history of dysentery and in families without a male adult. Epidemic disease remains a possibility in many parts of the world, often in association with civil disruption and migration of large numbers of people, as occurred in Central Africa in the mid-1990s.[69]

5.3. Geographic Distribution

Shigella is worldwide in distribution. The incidence of infection in different regions will vary, of course, with the level of sanitation and exposure. *S. sonnei* has become the

main isolate in industrialized countries, whereas *S. flexneri* remains the most common isolate in developing countries. *S. dysenteriae* 1 is now endemic, and occasionally epidemic, in the poor countries of Asia, Africa, and Latin America. *S. boydii* remains relatively uncommon everywhere (<1–2%) except in the Indian subcontinent. In the United States, the highest *Shigella* isolation rates are from the Southwestern states, where the relative proportion of *S. flexneri* is higher than elsewhere in the country. This may be related to the proximity to Mexico and the cross-border flow of migrant workers. Infection with *S. dysenteriae* 1 in developed countries, and to a lesser extent *S. flexneri*, is related to travel to endemic developing countries.[70]

5.4. Seasonal Transmission

Seasonal patterns of shigellosis are common, but differ in timing around the world. In the United States peak incidence of both *S. sonnei* and *S. flexneri* infection occurs in summer and early fall, with a delay of around 6 weeks from the peak in ambient temperature in Massachusetts.[71] In both urban and rural Bangladesh, *Shigella* isolation peaks in the post-monsoon season of December and January and again in the hot, dry premonsoon period of April and May. In nearby Calcutta, India, the incidence of shigellosis is highest during the monsoon season itself, while in Calabar, Nigeria, the peak occurs during the dry season. In Egypt and among Bedouins in southern Israel peak incidence is during the early summer months of June and July. In contrast to the Bedouins, Jews with a Western lifestyle living in southern Israel experience summer and winter peaks, while in nearby Saudi Arabia a winter peak for *S. flexneri* has been reported. In Guatemala, the illness tends to peak just before and in the early weeks of the rainy season. In some locales, there is no clear seasonal peak, and cases occur all year round.

A variety of explanations, none entirely satisfactory, have been offered for the different temporal patterns and seasonal transmission observed. Explanations for summertime peaks in temperate climates include increased social activity of children when school is out of session, which may promote person-to-person transmission and perhaps longer survival of organisms in the environment, although desiccation is not good for viability. On the other hand, summertime peaks during the hot, dry season in tropical climates have been explained by decreased availability of water, making it more likely that contaminated water is used and because the limited availability of water has a negative impact on personal hygiene. Peaks before or during the rainy season have been explained, respectively, by seasonal worsening of nutritional status before the harvest and by increased contamination of water supplies due to water washing feces deposited in unsanitary manner on the ground.

5.5. Age

Endemic shigellosis is a childhood disease in both developed and developing nations, whereas epidemic shigellosis due to a newly introduced strain affects all age groups.[67] But, even where shigellosis is highly endemic, neonatal shigellosis, generally originating from an infection in the mother, is rare. This is attributed to the high prevalence of antibody in breast milk among mothers in endemic settings.[72] Breast-feeding has been shown to be protective against disease among infected infants,[73] and perhaps this is why neonatal infection is often transient and asymptomatic in developing countries but systemic and severe in bottle-fed neonates in the United States.[74] Exclusive breast-feeding also reduces the likelihood of infection through contaminated weaning foods. In countries with a high endemicity of disease, shigellosis rates increase dramatically in the 3 months following cessation of breast-feeding, with an adjusted odds ratio of 6.6 (95% confidence interval, CI = 2.19–14.6, $p < 0.001$).[75] Although so-called weanling diarrhea has traditionally been attributed primarily to the risks associated with contaminated weaning foods, the addition of food supplements while breast-feeding is maintained does not increase shigella infection rates over the first 3 months of supplementary feeding, with an odds ratio of just 1.2 for addition of food supplements if breast-feeding continues (95% CI = 0.4–3.0).

However, neonatal shigellosis in developing countries may not be as benign a problem as first believed. In a retrospective 5-year review of admissions from 1984 to 1989 to an international diarrhea treatment center in Bangladesh, 4,741 patients with culture-proven shigellosis were identified.[76] Of these, 169 (3.1%) were 3 months of age or younger, of whom 19% were neonates under 1 month of age. Records were available on 159 subjects ≤3 months of age, including 30 (18.9%) neonates, and were compared with findings in 156 children 1–10 years of age, with a mean age of 27 months. Infants were much less likely to have bloody diarrhea (17.2 vs. 57.3%), fever (32.7 vs. 58.6%), pedal edema (3.8 vs. 27.2%), or rectal prolapse (0 vs. 8.3%) on admission; however, they were significantly more likely to be moderately to severely dehydrated (59.9 vs. 31.1%) or to have abdominal distension (19.5 vs. 10.1%) or bacteremia (12 vs. 5%), and were twice as likely to die (16.4 vs. 8.2%). Multivariate analysis showed that concomitant gram-negative bacteremia, ileus, decreased bowel sounds, or severe hyponatremia or hypoproteinemia were significantly

associated with fatal outcome. Interestingly, although three fifths of the cases in each age group were due to *S. flexneri*, neonates had a significantly greater prevalence of *S. boydii* (21.4 vs. 6.3%) and *S. sonnei* (7.6 vs. 1.3%), while a smaller proportion had *S. dysenteriae* 1 (9.4 vs. 31.5%).

Shigella infection rates increase in endemic developing country settings after weaning, when poor diets and frequent infections combine to adversely affect host nutritional status. In Bangladesh, the highest incidence of Shigella bacteremia and death is in infants under the age of 1 year, and the associated risk factors are weaning and malnutrition.[77] During the epidemics of dysentery due to *S. dysenteriae* 1 in the past two decades, the very young and elderly have experienced the most severe illness and the highest mortality rates.

Prevalence diminishes after the first few years of life, with a decrease in severity of illness and a relative increase in the proportion of mild and subclinical infections. As overall prevalence of infection in the community diminishes, as in developed countries, there is an apparent shift in the age-specific peak to a somewhat older age group. In the United States, the proportion of isolates in the under 5-year-olds decreased from around 47 to 37% from 1967 to 1988, while the proportion occurring in those over 20 years of age increased from 17.3 to 37.1%, especially after 1974.[53] A further decrease to 21% of confirmed *Shigella* infections in under 5-year-olds was noted in the surveillance years 1996–1998 in the population covered by the FoodNet active surveillance system, although this age group represented only 7% of the covered population.[78] The proportional rise in shigellosis among adults was 7-fold greater than the proportional increase in the size of the US population over 20 years of age during the same period of time, and approximately three quarters of the adult patients were 20–39 years old.

5.6. Gender and Sexual Practices

Age-specific attack rates for *Shigella* infection in children and for the whole US population show no gender differences. However, women account for significantly more infections among young adults. In the United States in 2004, the percentage of infected females was significantly greater than in males in the age groups 20–29 years (64.4%) and 30–39 years (53.4%), presumably reflecting their greater contact with young children. In contrast, there is a male preponderance for *S. flexneri* infection, particularly in the age groups 30–39 years (64.6%) and 40–49 years (65.6%). This is consistent with sexually transmitted infection among men who have sex with men and with the previously reported predominance of *S. flexneri* infection in this group. [79] From 1977 to 1991 there was an increase in the median age for *S. flexneri* infection from 9 to over 20 years, peaking in 1984, and then tending downward in more recent years, without a change in the median age for *S. sonnei* infections (around 7 years). This may indicate a return to the pre-1975 trend of diminishing *S. flexneri* prevalence, perhaps reflecting changing sexual practices among this group of young men. Outbreaks of *S. sonnei* among men having sex with men have been noted in the United States, Canada, Europe, and Australia.[80,81]

5.7. Race

There is no evidence that race per se affects susceptibility to the disease. Genetic factors, such as HLA-B27 antigen, clearly predispose to joint involvement and postdysenteric Reiter's syndrome; this may explain the frequency of these complications in Scandinavia, where the prevalence of histocompatibility antigen B27 in the population is relatively high.

5.8. Occupation

Certain occupations may increase exposure to shigellae. Notable examples are the increased incidence of shigellosis in women caring for young children at home. Teachers in day-care centers are also at greater risk than the general population,[82] and theoretically, so are attendants in custodial institutions for the mentally retarded, clinical microbiology laboratory technicians, and health care workers.[83] Although at risk, the lack of a striking incidence of shigellosis among laboratory technicians and health care workers suggests that good personal hygiene and ready access to clean water for washing is effective.

5.9. Occurrence in Different Settings

Shigellosis can readily spread among individuals living in close quarters where personal hygiene and environmental sanitation are inadequate. The 1994 epidemic among refugees fleeing Rwanda is evidence of this.[84] Bacillary dysentery has been a classic problem of armies at war. When US military personnel were sent to Somalia in 1992–1993 to safeguard emergency feeding programs, the diarrheal disease rates were unexpectedly low; however, *Shigella* was isolated from 33% of those affected.[77] More recently, 20% of marines, participating in the opening of the Iraq war in 2003, with etiologically proven diarrhea were infected with *Shigella*, second only to noroviruses.[85] Another setting in

Table 5. Secondary Attack Rates Among Household Contacts of Shigellosis Patients

	Secondary attack rates	
Age of exposed	*S. flexneri*	*S. sonnei*
Child	42	42
Adult	14	20

which *Shigella* outbreaks occur is aboard ships, because close contact among people is unavoidable and common meals are taken.[86] Outbreaks are much less common on commercial airplanes, and unless there are multiple cases among an identifiable group, may be difficult to identify.[87]

Introduction of illness into the household often results in intrafamilial spread, and the family should therefore be considered an epidemiological entity in this instance. The secondary attack rate is greater in children than in adults (Table 5) and infection is more likely to be symptomatic in the young as well. Nosocomial spread of shigellosis in a newborn nursery can result in serious systemic illness, shock, and death.[88]

5.10. Socioeconomic Factors

Endemic shigellosis is associated with poverty, crowding, poor sanitation, water sources inadequate in quantity and quality, and malnutrition.[65–67, 75, 76, 89] Prevalence of *Shigella*-specific antibodies, detected with specific ELISAs for serum *Shigella* lipopolysaccharides and invasion plasmid antigens, is inversely related to socioeconomic status.[5,88] Thus, water availability, water quality, sanitary disposal of feces, and control of flies each affect the infection rate individually. Controlling flies using a simple baited trap can reduce the prevalence of shigellosis by as much as 85% (Table 6).[90] However, these factors usually occur together in impoverished populations, and in general the impact of any single intervention may be limited. Pre-existing malnutrition exerts additional adverse effects and is associated with more severe illness and sequelae.[91] Bottle-feeding young infants where malnutrition is common will considerably worsen the situation, whereas breast-feeding appears to be protective until full weaning occurs.[92]

Table 6. Efficacy of a Simple Baited Fly Trap on Shigellosis Rates in Military Camps[a]

Parameter	Intervention camps	Control camps	"P" value	% Efficacy
Fly count	4.20	11.7	0.024	64
% Diarrhea	14.60	25.2	0.146	42
% *Shigella* isolated	0.06	4.0	0.015	85
% *Shigella* seroconversions	11.90	49.5	0.024	76

[a]Data from Cohen et al.[89]

6. Mechanisms and Routes of Transmission

Shigella, like the typhoid bacillus, is highly host adapted to humans and may, in addition, naturally infect a few nonhuman primates as well. For this reason, more than 99% of all *Shigella* isolates in the United States are from human sources, and the route of transmission is almost invariably traceable to an infected human. Transmission is a circle from the stool to the mouth and back to the stool again. While intervening vehicles can be involved, such as food and water, shigellosis is most frequently spread by person-to-person contact. Large numbers of shigellae (10^5–10^8) are present in stools of clinical cases, whereas healthy carriers have less than 10^3 organisms/g feces. Even under epidemic conditions, epidemiological evidence favors contact spread.[84] This is encouraged by conditions of poverty, crowding, and poor sanitation, exemplified by the situation in camps for refugees, which facilitate the transfer of the small inoculum needed to initiate the infection. Contact with a recent case of dysentery is one of the prominent risk factors in developing countries; the index case within households is most often a young child under the age of 10 years.[78] Interestingly, while the use of pit or other protected latrines may not be associated with a reduction in *Shigella* transmission (odds ratio = 0.96, CI 0.43–2.15), use of a hanging latrine in which feces are discharged directly onto the ground or into a body of water can be associated with a significantly increased risk for infection (odds ratio = 1.42, 95% CI: 1.02–1.98, $p < 0.05$).[93]

Organisms, of course, can find their way to food or water sources and thereby initiate common-source epidemics.[94,95] While *Shigella* is not the most frequent pathogen identified in foodborne outbreaks, the list of documented vehicles and settings for such common-source outbreaks has been increasing over time. Foodborne *Shigella* infection is commonly traceable to an infected food handler; raw vegetables are the most common vehicle.[9] Large outbreaks have also been traced to commercially prepared shredded lettuce, and imported iceberg lettuce was the source of an outbreak in Norway, with 110 proven cases of *S. sonnei* spread via salad bars.[96,97] Thousands of individuals in Norway and other countries were undoubtedly exposed to the tainted lettuce as well, with an unknown number of unreported cases. Newly reported and different vehicles include interesting items such as moose soup in Alaska and raw

oysters along the Texas Gulf coast.[98,99] In the latter outbreak, an infected crew member of an oystering boat may have contaminated the oyster beds, since the toilet facilities aboard the ship consisted only of 5-gallon pails that were dumped overboard directly into the harvesting area. Globalization of the food supply has resulted in outbreaks of *S. sonnei* infection in Denmark and Australia due to contaminated baby corn produced in Thailand.[100]

Any mass gathering at which centrally prepared food is served can be associated with outbreaks of *Shigella* infection, especially when toilet and hand-washing facilities are primitive or limited. For example, a large *S. sonnei* outbreak occurred in 1988 at an outdoor 5-day summer music festival, affecting over 3,000 persons.[101] Major factors in this outbreak were the large number (>2,000) of volunteer food handlers preparing communal meals, limited access to soap and running water for hand washing, and a documented small outbreak of shigellosis among the staff before the festival participants arrived. Transmission from staff to attendees likely occurred early but was amplified by a large common-source outbreak due to contamination of an uncooked tofu salad served on the last day. Onset of illness peaked 2 days later, after the festival ended.

Another large outbreak happened in 1987 at a mass gathering in a national forest, where over 50% of almost 13,000 attendees were infected with *S. sonnei* transmitted via multiple routes, including food, water, and person-to-person spread.[102] When the participants returned home further dissemination of the outbreak strain followed, with at least three additional confirmed outbreaks linked to the first by the unique antibiotic-resistance pattern, phage type, and plasmid profile of the outbreak strain. Similarly, high attack rates were documented in a foodborne outbreak at summer camps for mentally retarded and developmentally disabled persons.[103] During the same period of time, nearby camps for normal youngsters recorded no shigella outbreaks.

Surveillance for waterborne disease outbreaks associated with drinking water in the United States from 1971 to 1994 reveals year-to-year variation in the number recorded.[104] Prior to 1986 there were 20–50 outbreaks identified per year, but this number was reduced to 10–20 in the subsequent years. Many different pathogens, including bacteria, protozoa, and viruses have been isolated; *S. sonnei* is one of the most common bacterial agents. Recreational water, including pools, ponds, and lakes, can be directly contaminated by feces deposited by human or animal carriers (although this is not relevant for *Shigella*) or from sewage leaking into the water and has increasingly been implicated in shigella outbreaks.[105]

In 1991, a particularly interesting outbreak of bloody diarrhea and hemolytic–uremic syndrome due to *E. coli* O157:H7 and *S. sonnei* occurred at a lakeside park near Portland, Oregon.[106] No association with food or beverage consumption was detected; however, case–control studies strongly associated illness with swimming, especially among individuals reporting prolonged time in the water and swallowing of lake water. Although neither outbreak organism was recovered from the lake, enterococci counts indicated significant fecal contamination. A similar outbreak involving 65 persons occurred at a park pond in Michigan in 1989.[107] Increased risk was associated with swimming with the head submerged. There were no detectable defects in the park's toilets or sewage disposal system, implicating bather contamination of the waters. Waterborne transmission is not restricted to swimming in contaminated water; even wind surfing on contaminated waters can lead to infection.[108]

In developing countries, a combination of contact and waterborne spread can contribute to prolonged, smoldering epidemics that last for months rather than weeks, until the population of susceptibles is exhausted. In the Central American *S. dysenteriae* 1 epidemic of 1969–1972, waterborne spread within some communities is suggested by the clustering of cases in place and time, the presence of multiple index cases in families, and the relationship between defective water distribution and illness. However, the occurrence of the disease in widely separated places that do not share food or water supplies also indicates the likely role of the human carrier in spread. In the initial Bangladesh outbreak of epidemic *S. dysenteriae* 1 among the population of a semi-isolated coral island, the introduction of the organisms into this insular community was undoubtedly from an infected human, and the early slow spread of disease is probably explained by initial person-to-person contact. Later spread was most likely caused by contamination of water sources from the excreta of the slowly increasing number of patients, and the epidemic terminated when chlorination of water was begun.

A variation on the mechanism, but not the route, of transmission has been demonstrated in recent years among homosexual men engaging in anal–oral sex practices. Severe *Shigella* sepsis has been reported in patients with acquired immunodeficiency syndrome (AIDS).[109] Sexually transmitted shigella can be associated with simultaneous transmission of other enteric pathogens, such as *Giardia lamblia*.

7. Pathogenesis and Immunity

7.1. Pathogenesis

Shigellosis is the clinical manifestation of a complex interplay between the microorganism and the host.[16] This

process appears to take approximately 2–5 days from the initial entry of the organism via the mouth to clinical disease, based on experimental human infections with *S. flexneri* 2a and *S. dysenteriae* 1.[19] Surprisingly, the clinically more virulent *S. dysenteriae* 1 strain has a longer mean incubation period than *S. flexneri* 2a under these conditions.

A sufficient inoculum must survive gastric acidity and other intestinal defense mechanisms to initiate infection. The mechanism for acid resistance has already been discussed. Human shigellosis is clearly a colonic process that results in bloody diarrhea and dysentery. Experimental shigellosis in the rhesus monkey provides evidence of involvement of both the proximal small bowel and the colon.[110] The small bowel could be the source of or contribute to watery diarrhea in shigellosis, but this remains conjecture in humans. Organisms ultimately reach the colon where they cause an invasive bacterial colitis, resulting in the typical bloody diarrhea or dysentery of shigellosis.[111] This process is now believed to be initiated by invasion of bacteria across the intestinal M cells. When the organisms reach the lamina propria they are ingested by phagocytic cells, leading to the production of cytokines, such as interleukin 8, which initiates an inflammatory response. The subsequent migration of leukocytes between epithelial cells disrupts the tight junctions and allows large numbers of *Shigella* to reach the basolateral membrane of the epithelial cells, where they are able to invade directly into enterocytes. The subsequent contiguous spread of infection from one epithelial cell to another leads to focal areas of cell damage, ulcer formation, and bleeding and further inflammatory cell exudates into the lumen, the hallmarks of dysentery.

High levels of circulating LPS are present in patients with *S. dysenteriae* 1 infection and lower levels are common in those with *S. flexneri*, even in the absence of documented bacteremia. The frequency of endotoxemia in shigellosis suggests a broader role for LPS in pathogenesis of the disease, for example, the induction of proinflammatory cytokines. However, Shigella-induced apoptosis of macrophages, mediated by caspase-1, is itself sufficient to induce the release of proinflammatory cytokines.[112] Detectable fecal levels of certain cytokines have been reported in children with *S. dysenteriae* 1 and *S. flexneri* infection.[113,114] Although no differences in stool or serum levels of tumor necrosis factor (TNF) were observed between patients with uncomplicated infections and those with hemolytic–uremic syndrome (HUS), leukemoid reaction, or severe colitis, serum interleukin 6 (IL-6) was significantly higher in the children with complicated illness.[114] In a more elaborate study, an increase in the number of cytokine-producing cells was observed in cryopreserved rectal biopsies from adult patients with either *S. dysenteriae* 1 or *S. flexneri* infection.[115] IL-1β, IL-4, IL-10, and transforming growth factor (TGF)-β1–3 responses predominated; however, there was a universal response of all cytokines studied, including Th-1 and Th-2 agonist and antagonist cytokines (IL-1α and IL-1β, IL-1 receptor antagonist, TNF-α, IL-4, IL-6, IL-8, IL-10, gamma interferon, and TGF-β1–3). Cytokine-producing cells persisted in similar number when studied 30 days after onset of acute infection. The number of IL-1, IL-6, IFN-γ, and TGF-β-producing cells was significantly and directly related to severity of inflammation, assessed by histological criteria. These cells were also greater in patients with *S. dysenteriae* 1 infection compared to *S. flexneri* infection.

The results are consistent with quantitative observations on inflammatory cells and erythrocytes in the stool, in which patients with *S. dysenteriae*, *S. flexneri*, and *S. boydii* infection are more likely to have both large numbers of fecal leukocytes and erythrocytes than patients with *S. sonnei*, and the number of leukocytes is highest in *S. dysenteriae*.[116] It is likely that severe inflammation underlies pathogenesis of ileus and toxic megacolon in shigellosis. Further evidence for a role of cytokines in pathogenesis has been provided by experimental studies in the rabbit ligated ileal loop model, in which intravenous infusion of IL-1 receptor antagonist prior to initiation of *S. flexneri* infection within the loop dramatically decreases bacterial invasion of the tissue, and mucosal inflammation and tissue destruction.[117] Production of pyrogenic proinflammatory cytokines can explain clinical symptoms such as fever and anorexia. The severity of illness, including consequences of inflammation such as bloody diarrhea and dysentery, is directly related to the fever response.[118]

The factor(s) inciting the watery diarrhea that initiates clinical illness and predominates in milder episodes, including most cases of *S. sonnei* infection, remains uncertain. The suggestion that this is a small bowel process due to *Shigella* enterotoxins has remained an attractive hypothesis, since watery diarrhea temporally precedes bloody diarrhea–dysentery as the organisms move down the intestinal tract toward the colon. Experimental primate infections provide a cogent but not conclusive argument for a small bowel phase of symptoms and the role of enterotoxins.[110] However, neither Shiga toxin (which is not only cytotoxic but also causes net secretion across small bowel mucosa by targeting and inhibiting villus cell sodium absorption but not crypt cell chloride secretion,[119]) nor the newly described ShET toxins[42,43] (which are weakly secretory in rabbit bowel by an as yet unknown mechanism) are present in all *Shigella* spp.

Fever is typical in shigellosis and a consequence of inflammation and the production of pyrogenic cytokines.

A number of systemic complications, probably related to cytokine production, occur in shigellosis. Bacteremia, most commonly encountered in infections due to *S. dysenteriae* 1 or *S. flexneri*, occur in around 4–5% of hospitalized patients in developing countries. Bacteremias due to the common enteric gram-negative rods are detected as often as are *Shigella* and are likely the result of tissue damage. In Bangladesh, bacteremia occurs primarily in children under 1 year of age and is significantly associated with malnutrition. When the sensitivity of *Shigella* to the serum bactericidal response has been studied, *S. dysenteriae* and S. *flexneri* are the most serum sensitive rather than serum resistant, as might be expected for bacteremia isolates.[120] However, patients with *Shigella* bacteremia had a 43-fold decrease in serum bactericidal activity compared to normally nourished nonbacteremic controls. Therefore, acquired defects in host defenses, for example, diminished complement activity or impaired antibody production associated with malnutrition, seem to be the most likely explanation for the bacteremia.[121]

Leukemoid reactions occur most often in *S. dysenteriae* 1 infection, often associated with HUS, a syndrome of hemolytic anemia and renal failure secondary to microangiopathic injury of endothelial cells.[122] Shiga toxin appears to play a significant role in pathogenesis of HUS since it is associated with toxin-producing *S. dysenteriae* type 1 and Stx-1- and Stx-2-producing *E. coli*. These toxins are known to target, damage, and create a pro-thrombotic state in glomerular endothelial cells, at least in experiments in vitro, especially when endothelium is preactivated by LPS or inflammatory cytokines, which increase expression of the glycolipid toxin receptor.[123,124] Toxic megacolon is an uncommon but severe problem, reflecting extreme inflammatory colitis and may result in perforation of the gut, peritonitis, and systemic sepsis.[123, 124]

Neurological manifestations in shigellosis, most commonly seizures, have been considered a variant of febrile seizures seen in young children with rapidly rising temperature due to other causes. No specific neurotoxic factors have been identified in *Shigella*. Although Shiga toxin has long been known as Shiga neurotoxin,[2] there is no evidence of a neurotoxic effect that might lead to seizure activity. There is at present no mechanism to explain the toxic encephalopathy sometimes observed during *Shigella* infections, most commonly *S. flexneri*.[125] Among nine cases of shigellosis seen among US troops deployed to Saudi Arabia during the Gulf War of 1991, two presented with "pseudomeningitis," a previously described but uncommon presentation of *Shigella* infection, occurring especially in infants and children, and readily confused with acute bacterial meningitis.[126]

7.2. Immunity

Strong epidemiological evidence suggests that immunity to *Shigella* infections is acquired following exposure to the pathogen. First, shigellosis is primarily a childhood disease except when a new epidemic strain is introduced into a susceptible population. In contrast to endemic shigellosis, epidemics involve all age groups. Second, immunization with live oral vaccines confer protection against the strains included in the vaccine, but not for circulating strains not in the vaccine. Third, rechallenge of volunteers who previously had experimental dysentery with the same serotype demonstrates serotype-specific protection.

These observations show that type-specific immunity develops after exposure to *Shigella*, directed primarily to serotype-specific LPS antigens. No other surface or secreted microbial component has been shown to be capable of inducing protective responses, although many of these constituents are antigenic. Antibody to LPS, which may mediate this protective response, is present in humans in increasing prevalence with increasing age, and titers rise more rapidly in endemic regions of the world where infants and children are more likely to encounter the organisms early in life.[127,128] It has been suggested that serum IgG antibody, in high enough titer, may be secreted into the intestinal lumen and, if in high enough titer, may be protective in the gastrointestinal tract.[129] This is the basis for the attempt to develop new generations of systemic killed vaccines. During clinical shigellosis, serum proteins leak across the intestinal mucosa in high concentration and sufficient amounts to result in a protein-losing enteropathy.[130] The presence of IgG antibody and leukocytes in the gut exudate should, at least theoretically, contribute to antibacterial local defense responses in the mucosa and the lumen. However, the quantity of serum proteins in stool, measured by levels of α_1-antitrypsin, and the number of stool leukocytes are also related to the severity of the illness and is greater in *S. dysenteriae* 1 infection compared to shigellosis due to other species of *Shigella*.

Serum anti-Shiga toxin antibody develops in the course of *S. dysenteriae* 1 infection. Its role in host defense has not been clear, since primates immunized with toxoid develop high titers of neutralizing antibody but are not protected from oral challenge with virulent organisms. Thus, they develop classic primate shigellosis, a severe and frequently lethal diarrhea and dysentery. Pre-existing serum anti-Shiga toxin antibody, however, could protect against the systemic microangiopathic pathology resulting in HUS or thrombotic thrombocytopenic purpura in either *S. dysenteriae* 1 or Shiga toxin-producing enterohemorrhagic *E. coli* infections. Experimental studies in animals demonstrate this effect of anti-Shiga toxin antibody. Infection with ShET-1 or -2

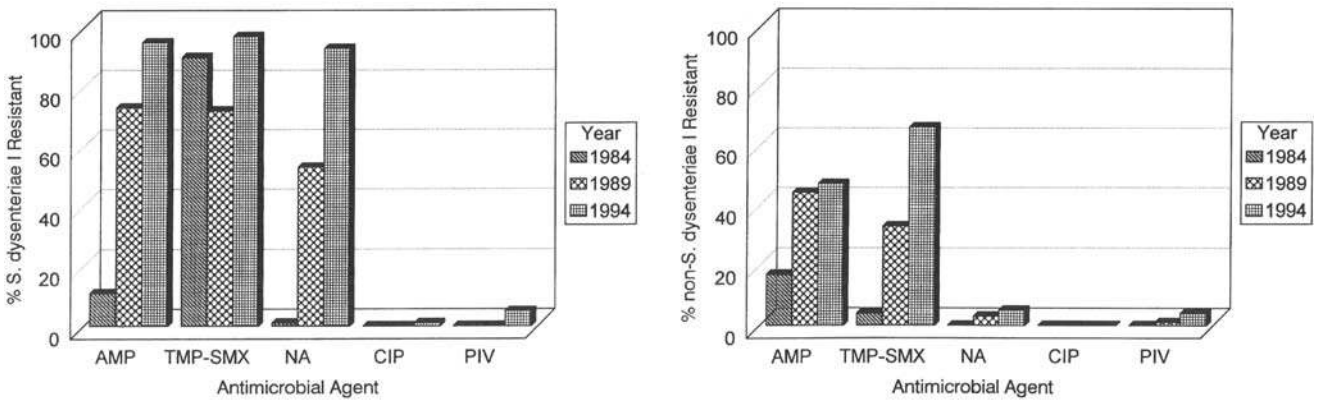


Figure 1. Antimicrobial resistance patterns of *S. dysenteriae* type 1 (*upper panel*) and other *Shigella* species, predominantly *S. flexneri* (*lower panel*), to the most commonly used drugs in Dhaka, Bangladesh, in 1984, 1989, and 1994. Following the development of resistance by *S. dysenteriae* type 1 to ampicillin (AMP) and trimethoprim–sulfamethoxazole (TMP-SMX), there was a marked increase in the use of nalidixic acid (NAL), with a marked increase in the prevalence of NAL resistance. At this time, nearly all isolates are susceptible to ciprofloxacin (CIP) and pivamdinocillin (PIV). Resistance to AMP and TMP-SMX also has increased among other *Shigella* spp., but not as dramatically, and most isolates remained susceptible to NAL, CIP, and PIV in 1994.

enterotoxin-producing *Shigella* spp. induces specific circulating antibody. The potential role of these antibodies in control of disease during acute infection or in protection from subsequent episodes is unknown at this time; however, circulating antibody may not have an impact on events at the epithelial cell luminal surface.

8. Patterns of Host Response

In fact, most clinical shigellosis is intestinal, spanning a spectrum from watery diarrhea to bloody diarrhea or dysentery. The dysentery syndrome is the most severe end of the spectrum. A clear-cut dose–response can be seen in adult volunteers experimentally infected with known numbers of bacteria (Table 1).[9] Some individuals remain asymptomatic, due to the presence of pre-existing immunity (especially in endemic regions) and the typical small inoculum size (especially in previously unexposed individuals in nonendemic regions). However, the initial postweaning exposure to *Shigella*, wherever and whenever it occurs, will more likely result in symptomatic disease. Extraintestinal manifestations involving the central nervous system (such as seizures, nuchal rigidity, meningismus, and delirium), the blood (bacteremia, leukemoid reactions, thrombocytopenia, microangiopathic hemolytic anemia), the kidney (hyponatremia, uremia), the eye (keratoconjunctivitis, iritis), and the joints (nonsuppurative arthritis) are primarily associated with *S. dysenteriae* 1 and *S. flexneri* infections and are uncommon in the United States where *S. sonnei* predominates.

Postdysenteric Reiter's syndrome (iritis, urethritis, arthritis) is usually associated with a host expressing HLA-B27 antigen and primarily a consequence of *S. flexneri* infection. Joint manifestations in such genetically prone subjects, once initiated, are often chronic and destructive. Rarely patients exhibit metastatic infection of skin, wounds, the lungs, the joints, the urinary tract, or the meninges.

8.1. Clinical Features

The initial manifestations of acute shigellosis, fever, malaise, abdominal pain, and watery diarrhea are not pathognomonic for shigella infection. Even the presence of fever cannot distinguish between *Shigella* infection and rotavirus or bacterial infections such as enterotoxigenic *E. coli,* which are often associated with low-grade fevers. Suspicion of *Shigella* increases as the disease progresses with the appearance of blood and mucus in the stools. The rectum and sigmoid area are the most severely affected; however, the entire colon may be involved in very severe infections. Inflammation and ulceration result in tenesmus and frequent passage of small-volume stools that often consist of only blood and mucus (Figure 2), characteristic of dysentery syndrome. In very severe cases, toxic megacolon and/or colonic perforation may ensue. The more virulent the organism (*dysenteriae* 1 > *flexneri* > *boydii* or *sonnei*), the more likely and more rapid is the appearance of bloody diarrhea and more frequent is progression to dysentery after onset of watery diarrhea. In fact, the course may be so rapid in *S. dysenteriae* 1 infection

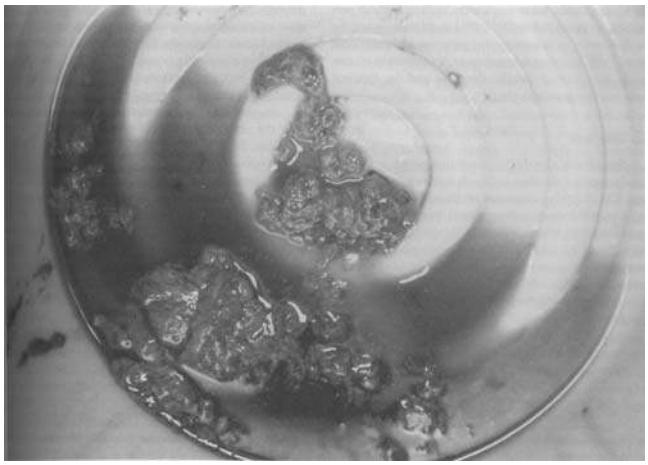


Figure 2. The appearance of the stool in most patients with shigellosis is, as shown here, a small-volume stool composed of fecal matter and bloody fluid, sometimes with mucus as well. Microscopic examination of the fluid phase reveals sheets of polymorphonuclear leukocytes and abundant erythrocytes. Passage of this type of stool is generally accompanied by abdominal cramps and tenesmus, and the three features together constitute a triad of findings that defines dysentery.

that it is hardly possible to distinguish the watery diarrheal phase at all.

In the United States, the increasing prevalence of *S. sonnei* has been associated with more mild watery diarrhea. In well-nourished subjects, the illness is generally self-limited in 5 or less days, without the need for antibiotics or other drug interventions. In the malnourished, however, *Shigella* can cause an acute fulminant infection associated with a number of complications, and continue on as a chronic relapsing disease, extending over months. At an international diarrhea treatment center in Bangladesh, where the majority of patients admitted to the inpatient unit are malnourished (mean weight for age = 59% of the NCHS 50th percentile), the fatality rate in 4,150 patients over a 5-year period was 9% (Table 7). In this population there was little difference in fatality rate for any *Shigella* species, including *S. sonnei,* suggesting that debilitation adversely affects the ability to defend against even the least virulent *Shigella* species.[89] Early deaths in shigellosis are generally due to septicemia, toxic megacolon, with or without perforation, or renal failure.[120, 123,124] Later fatalities are the result of complex interactions of infection and nutritional status.[121] In these circumstances, kwashiorkor or marasmic kwashiorkor develops and the terminal event is often another infection.

8.2. Diagnosis

Shigellosis should be suspected in febrile patients with bloody, mucoid diarrhea or the dysentery syndrome. The definitive diagnosis of shigellosis depends on identification of the organism in the stool. However, because early treatment can markedly shorten the duration and intensity of symptoms in more severely ill patients and impair the ability to culture the organism in vitro, presumptive diagnosis on the basis of clinical symptoms can be useful.[131] This is easiest for patients in endemic areas, patients infected during outbreaks, and those with classical dysentery. For example, in rural Bangladesh prospective community-based studies have shown that bloody, mucoid diarrhea accounts for two fifths of diarrhea episodes and two thirds of fatalities, about half associated with documented shigellosis.[132] The presence of many leukocytes and erythrocytes in stool adds to the suspicion of shigellosis.[133] In contrast the ability to make a correct presumptive diagnosis based on clinical features alone in the more highly endemic regions of the United States is less feasible than it was in the past when the prevalence of *Shigella* infection was as high as 33%.[134] In rare instances, *Shigella* patients develop seizures, sometimes even before the diarrhea presents, and therefore may be thought to have meningitis. Examination of the cerebrospinal fluid is usually normal, and the correct diagnosis becomes apparent when the intestinal manifestations develop, the stool is cultured, and the organism is identified.

The increasing prevalence in the United States of infection with Shiga toxin-producing *E. coli* (STEC), including serotype O157:H7 and many others, has introduced a new diagnostic dilemma. Bloody diarrhea with seizures due to the microangiopathic systemic complications of Shiga toxins produced by these organisms can be confused with

Table 7. Outcome of Patients Infected with *Shigella* Admitted to a Diarrhea Treatment Unit, Dhaka, Bangladesh, 1983–1987

Species or serotype	Total admissions	Discharged alive	Left against advice	Transferred elsewhere	Died in hospital
S. dysenteriae 1	1,251	976 (78%)	136 (11%)	40 (3%)	99 (8%)
S. dysenteriae 2–10	132	112 (85%)	5 (4%)	2 (2%)	13 (10%)
S. flexneri	2,319	1,841 (79%)	211 (9%)	39 (2%)	230 (10%)
S. boydii	303	245 (81%)	24 (8%)	3 (1%)	31 (10%)
S. sonnei	145	119 (82%)	12 (1%)	2 (1%)	12 (8%)

shigellosis (Chapter 14). At the present time, it is known that antimicrobial therapy is useful in shigellosis, and early treatment appears to reduce the frequency of HUS[135]; it is not as certain in enterohemorrhagic *E. coli* infection. Selective culture methods using sorbitol–MacConkey agar and ELISA tests for Shiga toxins in stool are useful ways to diagnose *E. coli* O157:H7 and other *E. coli*.[136] DNA-based tests are being developed, including gene probes and polymerase chain reaction methods; however, as in other areas of diagnostic microbiology, these have not yet made it to the clinical laboratories.

In developing countries, where the vast majority of *Shigella* infections occur, laboratory facilities for standard microbiological isolation of the organism, let alone immunoassay tests, are often not available, and diagnosis is dependent on clinical evaluation, aided by stool microscopy for white blood cells. The major differential diagnosis, *E. histolytica*, is a much less common cause of dysentery in children and does not cause a purulent stool because the organism destroys neutrophils. In practice, all children with clinical dysentery in endemic regions should be presumed to have *Shigella* and treated accordingly. Under optimal conditions, the stool should be examined for motile amoebic trophozoites containing ingested erythrocytes to rule out amoebiasis. In adults, the incidence of amoebic dysentery is higher than in children, and a microscopic examination of stool is important to discriminate between the two illnesses.

9. Control and Prevention

9.1. General Concepts

Even though the infective inoculum for *Shigella* infection is small and person-to-person contact spread is common, it is surprising how effective simple hygiene measures can be used in control and prevention of shigellosis.[137] Hand washing and sanitary disposal of feces constitute the keystones of personal hygiene and will minimize the spread of infection within the household. Protection of food and water for drinking and cooking from potential contamination is a critical part of this approach. Within the community, the integrity and availability of the water supply and its protection from fecal contamination is important; this is most obvious when breaks in the system permit waterborne disease. Unsanitary latrines, which allow feces to be deposited directly on the ground or in a body of water, concentrate infectious agents and facilitate transmission and are no doubt worse than no latrine at all.[138] Programs to build latrines should focus on simple sealed latrines such as the VIP (ventilated improved pit) latrine and its modification for areas with a high water table to avoid contamination of groundwater.[139] Breast-fed infants are at lesser risk of symptomatic *Shigella* infection than are bottle-fed neonates, and good nutritional status may limit the extent of acute infection and the chronicity of established illness.[75]

Travelers to endemic regions of the world are at risk of enteric infection, including shigellosis, primarily from contaminated food or water.[140] Flies may contribute to the transmission of shigellosis in areas where they are plentiful and feces are not hygienically disposed of. Simple baited fly traps can be built locally for little cost and appear to be highly effective but are not required (Table 6).[90,137]

It is wise for physicians making the diagnosis of acute shigellosis in one family member, usually a young child, to consider the whole family to be at risk. However, younger contacts of the index case are more likely to develop symptomatic secondary infection than are adults.

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis and Treatment

Daily high-dose bismuth subsalicylate (BSS, PeptoBismol®) has previously been shown to be protective against bacterial causes of travelers diarrhea, including *Shigella*, for adult travelers to Mexico.[141,4] However, the protection is modest and does not warrant the large number of tablets that must be taken, the limit of 3 weeks prophylaxis because of the potential accumulation of toxic levels of bismuth, the lack of safety in children under 3 years, and the potential side-effects of "black tongue."[142]

There is widespread interest to prevent or treat enteric infections with probiotics, harmless commensal bacteria that can colonize the intestine and prevent pathogens from establishing infection or possibly favorably modify the host cell response to pathogens.[143] Experimental evidence in animal or cell culture models suggest that certain probiotics can modify the severity of *Shigella* infection. In an in vivo model of infection in rats, pretreatment with *Lactobacillus rhamnosus* and *L. acidophilus* significantly attenuated the inflammatory changes associated with *S. dysenteriae* type 1 infection.[144] Cultured intestinal epithelial cells exposed to *L. casei* followed by challenge with *S. flexneri* demonstrated a marked downregulation of inflammatory mediator gene expression, which could decrease clinical manifestations in a real infection.[145] Convincing clinical trials in humans have yet to be conducted, and whether or not probiotics can be used for prophylaxis in high-risk settings for shigellosis or would be additive therapy remains unanswered.

Recent emphasis on the role of micronutrient deficiencies, including vitamin A and zinc, on susceptibility and response to acute diarrhea has suggested the potential of

routine supplementation to prevent diarrhea and/or use of these nutrients as adjunct treatment.[146] Clinical data are limited, and often contentious, as in the case of vitamin A supplements to treat shigellosis in Bangladesh.[147,148] Zinc supplementation is reported to significantly shorten the duration of acute shigellosis, improve weight gain during recovery, and reduce the burden of diarrheal episodes for the next 6 months of follow-up.[149]

There is little information on the utility of prophylactic antimicrobials for shigellosis, and much to suggest this is inappropriate because of the rapidity with which antibiotic resistance develops. Prophylaxis with antibiotics would only make sense for short-term visitors to an endemic region, because in endemic settings, exposure to infection occurs much too frequently to make prophylaxis a viable consideration for the local population or even for resident expatriates. Because of widespread resistance to the usual drugs proposed for prophylaxis of diarrhea in travelers, the introduction of rifaximin, a non-absorbable rifamycin antibiotic, is being touted as the drug to use.[150] In a trial using experimental infection with *S. flexneri* 2a in human volunteers, rifaximin completely prevented diarrhea and dysentery (vs. 60 and 10% for the placebo control group).[151] The use of antimicrobial agents for prophylaxis could be considered in outbreaks, for example to prevent intrafamilial spread or reduce disease among contacts at risk, but the concern remains that excessive use would rapidly select for resistance. In confined outbreaks, such as newborn nurseries, standard methods of cohorting, implementation of strict sanitary measures, bacteriologic screening of medical staff, and judicious use of antimicrobials can bring an epidemic to a halt.[152]

Antimicrobial treatment of shigellosis should be regularly employed for dysentery, considered in bloody diarrhea, depending on clinical severity and the underlying health of the patient, and withheld in mild watery diarrhea. The latter recommendation is especially valid for management of otherwise healthy subjects in areas where *S. sonnei* prevails, since such infections are almost always self-limited without the use of antibiotics. Recently multidrug-resistant *S. sonnei* have emerged and are spreading globally,[100] and it is likely that resistance will continue to evolve as antibiotic use grows. It is necessary to know the prevalence of resistance and to monitor this over time in order to use antibiotics most effectively when appropriate. Quinolones are the best option for adults in most regions of the world and are increasingly seen as safe in children, despite concerns about an association with arthritis.[153] With more common use of quinolones, especially in developing countries where such drugs are readily available, there is a danger that resistance will emerge, and such strains are being detected in travelers returning to developed countries.[154] Parenteral cephalosporins have been a standby when oral drugs fail; however, the spread of extended spectrum cephalosporinases in *S. sonnei* auger poorly for the future. A large outbreak of dysentery due to resistant organisms has occurred in Taiwan.[155] Resistance to azithromycin in Bangladesh strains has been increasing, even though use of the drug is still limited due to cost.[45] Early treatment with effective antibiotics has been shown to reduce the frequency of one of the life-threatening complications of *S. dysenteriae* type 1 infection, hemolytic–uremic syndrome, associated with a decrease in the content of Shiga toxin in the stool.[135]

9.3. Immunization

There is no licensed vaccine for shigellosis at this time, but not because of a lack of effort to develop one. In fact, effective prototype vaccines have been prepared for oral use in humans, but none to date is both effective and safe.[156] Because acquired immunity is serotype specific, an ideal vaccine would have to do much better by inducing protection to multiple serotypes. Otherwise, a combination of vaccines inducing protection against single serotypes would need to be developed, which increases the cost of development and the cost of any effective product that emerges.

Although most modern approaches to vaccine development for enteric infection have concentrated on live oral attenuated vaccine strains or recombinant bacteria expressing protective antigens, there has also been a return to the testing of parenteral or oral killed vaccines or antigens. Parenteral immunization with killed organisms has never been shown to be protective in humans, although there has been success in animal models.[157] New constructs of *Shigella* LPS O-antigens conjugated to protein carriers induce IgG antibodies in both young children and adults that may be protective.[158,159]

Serum IgG antibodies reaching the lamina propria may prevent shigella invasion and growth in inflammatory cells, the release of cytokines, and the invasion of epithelial cells, thus attenuating infection at the earliest stages of pathogenesis. Time will tell whether this approach will succeed, although the presence of mild fever in 5–10% of volunteers suggests the reactivity may be a problem for regulatory approval in the United States.

Because of the role of Shiga toxin in producing the severe disease due to *S. dysenteriae* 1 and in the pathogenesis of systemic microangiopathic disease in this infection and in STEC, attempts to develop a safe toxin immunogen for active immunization or antibodies for passive immunization have been initiated.[160] Toxin is a complex protein consisting

of one biologically active A subunit and five B subunits involved in binding to host cells. Various groups are testing mutationally inactivated A subunit holotoxins, isolated recombinant B subunit, or synthetic peptides linked to protein carriers by conjugation or synthesized as recombinant fusion proteins. Promising results have been obtained in animal models; however, it will be a while before such vaccines are tested in humans. Passive immunization with anti-toxin to *E. coli* Stx-2 have been successful in preventing systemic complications in animal models.[161] These various products are being developed primarily for the prevention or treatment of STEC infections, but if effective they will surely be useful in reducing the severity and complications of *S. dysenteriae* type 1 infections.

10. Unresolved Problems

10.1. Epidemiology

Because shigellae are so host-adapted to humans and a few subhuman primates, there is an absolute need for constant passage of the organisms through these hosts. At first glance, it would seem improbable that the organism could survive this restriction when environmental sanitation and protected water supplies limit transmission by the water route. Nonetheless, we know that the organism persists under these conditions, indicating continual person-to-person or foodborne transmission. It is uncertain how in fact the organism does maintain itself, particularly during periods of low prevalence. The role and importance of asymptomatic carriers may be much greater than previously thought. The reasons for the major shifts in prevalence of *Shigella* species in different regions over time are also not known. The use of newer molecular methods to detect organisms and for typing isolates should help unravel many of the questions relating to the presence and transmission of infection in the community.

10.2. Pathogenesis

Spectacular progress has been made in defining the pathogenesis of *Shigella* infections since the application of modern molecular and cellular biology approaches. These studies have served to demonstrate how complex this process is, that multiple virulence genes are required, and that the host factors altered by the organism plays a major role in the development of clinical illness. Most progress has occurred in the mechanism of host cell invasion and in the regulatory mechanisms for inflammatory responses. We still know too little of the specific role and mechanism of toxins, including Shiga toxin and the Shigella enterotoxins Shet-1 and -2 in pathogenesis of local events in the gastrointestinal tract or in the causation of systemic pathology. Elucidation of the specific mechanisms involved is essential for the development of therapeutic and prophylactic measures.

Rational pharmacological manipulation of the secretory or inflammatory process in the intestinal tract is equally dependent on specific knowledge of the mechanisms involved. These take on particular significance when the problems of epidemiology and antimicrobial drug resistance are considered.

10.3. Antimicrobial Resistance

Transferable multiple antimicrobial drug resistance mediated by plasmid DNA was first discovered in *Shigella*, ushering in a dramatic rise in the prevalence of highly drug-resistant shigellae in Japan and subsequently elsewhere as antibiotic pressures led to selection of the plasmid-bearing organisms. Resistance clearly restricts the efficacy of antimicrobial therapy of acute shigellosis. The principal strategy in dealing with acquired drug resistance has been to develop new or modify old drugs to preserve antimicrobial activity. This merely buys time; the ultimate solution is to learn how to control transferable drug resistance in nature. If it were possible to remove these plasmids in vivo it would restore susceptibility to the drugs already available for use and known to be effective, to be inexpensive, and well tolerated. If we knew how to accomplish this it could have the added advantage of curing plasmids bearing essential virulence traits, rendering the survivors nonpathogenic. The theoretical barriers to this are enormous and are further complicated by the tissue- and cell-invasive characteristics of *Shigella*, which insure a protected reservoir of fully virulent and drug-resistant organisms unless the strategy is effective beyond the intestinal lumen. Even so, the ability to cure undesirable plasmids in nature could have important effects on the prevalence of fully virulent organisms excreted into the environment.

Other ways to approach these issues at a more practical level involve control and restriction in the use of antimicrobial agents. In the United States, where such drugs are dispensed only by prescription, physician and patient education could have a major impact; however, success in such programs has been limited. Even if this were accomplished the use of similar or identical drugs in agriculture to promote the growth of meat animals, which selects for resistance, could overcome the benefits of reducing antibiotic use in humans. In developing countries, despite the efforts toward essential national drug programs, there is no control over dispensing

antimicrobials, which are freely available on the market and are used indiscriminately. The need for legislation and effective agencies to monitor and control drug use comes into conflict with the enormous potential economic gains for both local and multinational drug manufacturers as well as local drug merchants, including pharmacists and untrained dispensers, and the problem of insuring that patients have access to the drugs they may need when access to medical care is so limited.

10.4. Clinical Complications

The pathogenesis of most of the clinical complications that occur in shigellosis is not well understood. Since certain of these complications, including microangiopathic hemolytic anemia, hyponatremia, and thrombocytopenia, occur almost exclusively in association with *S. dysenteriae* 1 infection, speculation has centered on the role of Shiga toxin produced by this organism. The discovery that Shiga toxin-producing *E. coli* cause similar systemic events in children in developed nations and the occurrence of outbreaks involving large numbers of individuals in the United States have stirred research interest and have led to increased support for research. Much remains to be done.

Toxic megacolon is another complication associated with significant mortality. It is logical to assume that the extent of the inflammatory changes is the major determinant of toxic dilatation, but little is known in fact. Therapy requires appropriate antimicrobials and general support with fluids, reserving surgery for those who do not respond or whose colon perforates. New data on the cytokine response in shigellosis suggest that these inflammatory mediators play a major role in inflammatory complications. Further studies on their role in pathogenesis are warranted, and the potential of new anticytokine strategies of therapy in this and other complications is an exciting new area for study.

In the case of Reiter's arthritis, there is a clear genetic predilection for HLA-B27-positive subjects. The mechanisms underlying this particular host response to the infection need to be understood. Therapy or, even better, prevention could then be considered. This is clearly important because of the chronic destructive joint disease initiated by the organisms.

Shigella infection is associated with the most severe nutritional consequences of all the diarrheal diseases. In developing countries, this malnutrition is strongly associated with mortality, and there is growing evidence that the mortality rate in patients in the months after discharge from the hospital after shigellosis is far greater than in those discharged after watery diarrhea due to *E. coli*, cholera, or rotavirus. Nutritional effects of the infection may account for this in large part. The relative importance and persistence of mucosal inflammation, systemic catabolic responses, and protein-losing enteropathy are uncertain, and there are no pathogenesis-based innovative approaches to prevention and treatment.

10.5. Vaccines

Because of unresolved questions of pathogenesis and the failure to develop acceptable vaccines, the question of prophylaxis is open to investigation. It is apparent that our current understanding of host defenses and the nature of the protective immune response in shigellosis is wanting. When success does not come easily with conventional approaches, it is time to stop and reason. In the present context, this means more studies to define the protection-eliciting antigens of the organisms and to find a way to present these to the host without toxicity or unacceptable side-effects to achieve the protection desired.

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CHAPTER 34

Evans' Infections of Humans: Staphylococcal Infections

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1. Introduction

Despite major advances in the medical arena, staphylococci remain important agents of infectious diseases in the human host. Although initially disregarded as contaminants in cultures, coagulase-negative staphylococci have recently gained great clinical significance and are now recognized as potentially virulent pathogens associated with significant morbidity and mortality.[1] The emergence of antibiotic resistance has also brought renewed attention to staphylococci. Methicillin-resistant *Staphylococcus aureus* (MRSA) is now considered a major public health problem both in the hospital and in community settings.[2–4]

2. Historical Background

Approximately 100 years ago, von Recklinghausen used the term "micrococci" to describe gram-positive cocci observed in diseased tissue and in pus from human abscesses. In 1881, Sir Alexander Ogston published extensive observations showing that a cluster-forming coccus was the cause of certain pyogenic abscesses in humans.[5] He subsequently named the organism *Staphylococcus*, derived from the Greek nouns *staphyle* (grapes) and *coccus* (grain or berry). He distinguished staphylococci from streptococci and produced disease in mice by subcutaneous inoculation of staphylococci recovered from pus. In 1884, Rosenbach was able to isolate staphylococci in pure culture and observed that staphylococcal isolates formed colonies of two different colors.[6] He named the orange colonies *S. aureus* and the white colonies *S. albus*. After making the distinction between *S. aureus* and the other staphylococci, Rosenbach showed that *S. aureus* was responsible for wound infections, while the other staphylococci were normally found on the skin as colonizers.

In the following years, staphylococci were recognized as causes of important and sometimes lethal infections, including surgical wound infections and pneumonia.[7,8] The discovery of the antibacterial activity of the mold *Penicillium* in 1928 by Fleming[9] and the subsequent production of penicillin gave scientists hope that all infectious diseases will be promptly eradicated.[10] For a period of time, staphylococcal infections became less important causes of nosocomial infections than gram-negative bacilli. However, the rapid emergence of resistant isolates of *S. aureus* just 1 year after penicillin was marketed was capable to reverse the tide and allow staphylococci to ascend to the forefront of infectious diseases.

3. Methodology

3.1. Sources of Mortality Data

Most staphylococcal disease is localized to the skin and is not fatal. Once infection becomes generalized and bacteremia occurs, mortality is great despite appropriate antibiotic therapy. Data on mortality from bacteremic staphy-

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lococcal infections have come from national surveillance[11] and from hospital studies[12–14] with comparable findings.

3.2. Sources of Morbidity Data

The incidence of all staphylococcal disease in the community can be assessed only indirectly, since minor disease may never come to medical attention.[15] The incidence of more serious disease, including bacteremia, can be estimated.[16,17] Staphylococcal disease is not part of the national reportable disease surveillance system. Hospital surveillance has provided reliable data on hospital-acquired staphylococcal disease[12,18,19] and on methicillin-resistant *S. aureus* disease in long-term care facilities.[20,21]

3.3. Surveys

Household- and population-based surveys have provided a wealth of data on community-acquired morbidity from staphylococci.[22,23] Hospital-based surveillance for staphylococcal disease, community and nosocomially acquired, allows for ongoing assessment of serious staphylococcal disease.[12,18,19] Hospital surveillance begins from laboratory cultures reporting staphylococci; hence, it may underestimate the incidence of disease when cultures are not taken. Typing of staphylococci in the laboratory permits identification of strains responsible for outbreaks or epidemics.[24]

3.4. Laboratory Diagnosis

Staphylococci can be recognized quickly and reliably on Gram stains of exudates from most clinical specimens. Their lack of fastidiousness permits their growth on ordinary laboratory media.[25] Selective media such as that containing mannitol and 6.5% NaCl have expedited recognition of staphylococci grown from swabs of throat, nose, or other contaminated areas and have permitted rapid evaluation of staphylococcal carriage by individuals. Once grown, *S. aureus* is identified by a positive coagulase test.

There are over 30 species of coagulase-negative staphylococci. Although many clinical laboratories do not routinely speciate coagulase-negative isolates, they can be distinguished by a variety of biochemical reactions. *S. epidermidis* accounts for the majority of clinical isolates, with *S. haemolyticus* and *S. hominis* next in frequency.[26] Antibiotic sensitivity of staphylococci as measured by a Kirby–Bauer disk technique is widely used,[27] although in selected instances, tube-dilution techniques permitting the determination of minimal inhibitory and bactericidal concentrations are more valuable.[28] Phage typing of staphylococci has permitted identification of specific strains and has allowed for the recognition of prevalent and epidemic strains. Molecular methods of typing, such as plasmid analysis or restriction endonuclease analysis of whole-cell DNA, are highly reliable in differentiating staphylococcal strains[29,30]; they are more widely available than phage typing. Strain identification by antimicrobial susceptibility is unreliable.

The serological diagnosis of *S. aureus* bacteremia has been evaluated.[31] Antibodies to a variety of staphylococcal antigens have been tested including peptidoglycan, teichoic acid, *S. aureus* ultrasonicate, whole *S. aureus* cells, alpha-toxin, lipase, and capsular polysaccharide. Whole-cell ELISA has been shown to be the most sensitive assay although all tests lacked specificity. Studies suggest that the presence of antibodies to *S. aureus* teichoic acid might indicate a chronic deep-seated infection, including endocarditis, chronic osteomyelitis, and septic arthritis, whereas patients with uncomplicated bacteremia, acute osteomyelitis, cellulitis, and meningitis frequently have negative titers.[32] However, these tests have not come into clinical use. Antibodies against an exocellular glycolipid antigen in coagulase-negative staphylococci have also been used to distinguish between staphylococcal infection around prostheses and aseptic loosening.[33]

4. Biological Characteristics of the Organism

Staphylococci are nonmotile, gram-positive cocci that may occur singly, in pairs, short chains, or clusters. The characteristic appearance is that of a "bunch of grapes." They are facultative anaerobes but grow better under aerobic than under anaerobic conditions. Staphylococci grow readily on blood and chocolate agar. Colonies of *S. aureus* measure 1–3 mm and typically produce a yellow to golden pigment due to the presence of carotenoids. Most other species are nonpigmented. Most strains of *S. aureus* produce hemolysis within 24 to 36 hours on horse, sheep, or human blood-agar plates. They usually produce catalase. Traditionally, they have been divided into coagulase-positive and coagulase-negative staphylococci. The following enzymes and toxins that are produced by staphylococci are often considered of importance in human virulence:

1. *S. aureus* produces two antigenically distinct enzymes: bound and free coagulase, both capable of coagulating human serum. Their role in infection is unclear, but the presence of this enzyme serves to distinguish the more virulent species, *S. aureus*, from other species.
2. Alpha-toxin causes direct damage to a variety of mammalian cell membranes including erythrocytes, leukocytes, and fibroblasts.
3. Leukocidin is an extracellular protein that acts on human polymorphonuclear leukocytes in vitro. It causes death of white blood cells, but its role in infection is unclear.

4. Enterotoxin: Most *S. aureus* strains produce one or more enterotoxins that are responsible for the syndrome of staphylococcal food poisoning. A smaller percentage of *S. epidermidis* strains produce enterotoxin; these have rarely been implicated in food poisoning.[34]
5. Exfoliative toxin: Staphylococci of phage group II produce a toxin responsible for the dermatologic manifestations of infection that go under the general heading of "scalded-skin syndrome."
6. Toxic shock syndrome toxins: The syndrome is mostly caused by toxic shock syndrome toxin-1 (TSST-1). However, other exotoxins and enterotoxins can also be involved.[35,36] While rare, toxic shock syndrome has also been reported to be caused by TSST-1-producing coagulase-negative staphylococci.[37]
7. Enterotoxins and TSST-1 bind to class II major histocompatibility antigens and to receptors on T lymphocytes. These toxins stimulate the release of interleukin-2 and tumor necrosis factor; uncontrolled release of these lymphokines may play a pathogenic role in the clinical syndromes caused by these toxins.[38]

S. aureus specifically binds a variety of human proteins and glycopeptides, including fibronectin and fibrinogen. This binding may be an important factor in the maintenance of the carrier state and the initiation of infection. Clinical isolates of coagulase-negative staphylococcus, from patients with clinical infections, appear to produce an extracellular polysaccharide ("slime factor") that enhances adherence to plastic or metal surfaces.[39] This slime layer also inhibits diffusion of antibiotics, impairing their ability to reach organisms.[40] In addition, the slime material interferes with the lymphoproliferative response to mitogens[41] and also alters polymorphonuclear functions such as decreasing responsiveness to chemotactic stimuli and inhibiting phagocytosis of coagulase-negative staphylococci.[42] Coagulase-negative staphylococci have the ability to erode into polyethylene catheters and prosthetic devices, which may also allow increased adherence of the organisms.[39,43] Catheter-adherent coagulase-negative staphylococci appear to possess survival mechanisms under adverse conditions (absence of conventional nutrients) that may be important in the genesis of occult foreign-body-associated infection.[44]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Staphylococci have long been recognized as a hospital problem, and the policy of routine ongoing surveillance for hospital-acquired staphylococcal disease is well justified.[45–47] *S. aureus* is the leading cause of postoperative wound infection and the second-most frequent cause of nosocomial pneumonia.[48] Coagulase-negative staphylococci are now the leading cause of primary nosocomial bacteremia, accounting for 29% of cases.[48] During the period 1980–1989, the rate of bloodstream infections due to coagulase-negative staphylococci in large teaching hospitals rose from 0.2/1,000 discharges to 1.8/1,000.[49] The widespread use of invasive devices, such as pacemakers, central venous catheters, and hemodialysis catheters has contributed significantly to the temporal increase in healthcare associated staphylococcal infections.[48]

Serious staphylococcal disease may be acquired outside the hospital. The prevalence of such infections has recently increased significantly. In particular, community-associated MRSA (CA-MRSA) has become a public health concern, with the USA300 strain being predominant in the United States (1, 2). Despite few data on incidence rates, the burden of CA-MRSA infections is significant. For example, a Texas-based study in children noted a 14-fold increase in the rate of CA-MRSA infections (3). Similarly among adults, the incidence of CA-MRSA varied from 15 to up to 74% (4).

5.2. Nasal Carriage

S. aureus may be carried by normal people at various body sites without causing disease. The nose is the principal site of carriage; cross-sectional studies reveal rates of 30–50% in persons outside hospitals and higher rates in hospital nursing personnel.[54,55] More recent studies have demonstrated an increase in the rate of nasal colonization with methicillin-resistant *S. aureus* (MRSA) both in the community and in hospital settings.[56,57] Longitudinal studies have shown persons to be persistent carriers (20%), intermittent carriers (60%), or persistent noncarriers (20%).[58] Skin carriage has been demonstrated in 85% of persons with atopic dermatitis, suggesting a strong predisposition to carriage.[59] *S. aureus* carriage in infants is determined by a combination of host, environmental, and bacterial factors. After the eighth week of life, the carriage rate declines as infants develop an immune response to *S. aureus* and spontaneously clear the colonization.[60]

Carriage may precede or indeed lead to disease in susceptible individuals.[61] Patients on continuous ambulatory peritoneal dialysis who are carriers had a significantly higher infection rate than noncarriers.[62] Other populations at risk include surgical patients,[63] hemodialysis patients,[64] HIV-infected patients,[65] and those with intravascular devices.[66]

5.3. Epidemic Behavior and Contagiousness

S. aureus can be highly contagious through direct contact with the large numbers of organisms present in lesions. The condition of the host clearly influences the frequency and severity of disease produced by staphylococci. Within a

household, any strain of *S. aureus* can colonize family members and cause disease sporadically or it may cause continuous and repeated episodes of infection.[2]

Over the years, epidemics of *S. aureus* infections have been recognized throughout the world.[67,68] Outbreaks have been reported in a variety of settings including the community[69] as well as hospitals,[70] long-term care facilities,[71] and outpatient clinics.[72] A wide variety of hosts can also be affected including immunocompetent individuals[73] and patients with chronic diseases and altered defenses.[74] Nosocomial outbreaks have frequently been ascribed to colonization of the nares and hands of health care workers.[75,76] Finland[77] and others[68] have suggested that the epidemic character of *S. aureus* may relate, in part, to genetic properties of the organism. The changing susceptibility and resistance of *S. aureus* to bacteriophages or properties of the phages themselves may create within *S. aureus* the changes required for enhanced virulence. The changing antigenic character and epidemic properties of *S. aureus* may thus be analogous to the changing influenza A viruses and their epidemiological virulence.

The epidemic potential of *S. epidermidis* has also been demonstrated. Several outbreaks have been described, particularly in surgical patients[78,79] and those with indwelling catheters.[80]

5.4. Geographic Distribution

Infections with *S. aureus* are worldwide in distribution.[77] While the penicillin-resistant phage type 80/81 caused infections worldwide, the more recent methicillin-resistant strains have been of several phage types or were nontypeable[67] and are now widespread in Europe and [81,82] the United States[83,84] and occur worldwide.[85]

Penicillin-resistant forms of *S. aureus* do occur in nature,[86] and penicillinase-producing strains from the pre-antibiotic era have been reported.[87] In contrast, there is no clear documentation of resistance to other anti-staphylococcal drugs prior to their clinical use.[88]

5.5. Age

Children have higher rates of carriage and persist as carriers more commonly than do adults.[89] Mortality from serious staphylococcal infection is age related, with higher rates in the very young and older age groups.[90,91]

5.6. Sex

Male gender was found to be a risk factor for *S. aureus* nasal carriage in preoperative patients,[92] although other studies have not shown the same association.[93] On the other hand, infection[94] and mortality[95,96] rates were similar for both males and females in several studies.

5.7. Race

No racial predisposition for *S. aureus* has been noted.

5.8. Occupation

Health care workers who come in contact with patients colonized or infected with *S. aureus* have higher rates of nasal carriage than persons without such contact,[97,98] and they may develop clinical disease from such carriage.[99]

5.9. Socioeconomic Factors

There is little to suggest that socioeconomic factors influence the occurrence of staphylococcal disease. Overcrowding, poor skin hygiene, or minor skin trauma may play a role in predisposing to *S. aureus* bullous impetigo, but the association is greater for streptococcal pyoderma.[100]

5.10. Other Factors

A variety of underlying conditions predispose to *S. aureus* infection.[72,94,101–103] Extremes of age,[94,104] recent influenza A infection,[105] exfoliative skin disorders,[106] autoimmune disorders,[107] diabetes,[94] chronic renal failure,[108] liver disease,[101] and intravenous drug abuse[109] are associated with an increased risk of infection. In addition to surgery,[102] vascular catheters,[110] and dialysis,[111] medical interventions that predispose to infection with *S. aureus* include insertion of prosthetic devices[112] and administration of corticosteroids and other immunosuppressive agents.[113] The use of antibiotics has clearly influenced the epidemiology of *S. aureus* infections.

Coagulase-negative staphylococci are part of the normal skin flora and are uncommon pathogens except in the presence of foreign bodies (prosthetic valves, CSF shunts, prosthetic joints, and intravenous catheters). Immunocompromised hosts and neutropenic patients appear to have an increased susceptibility to severe infections, such as bacteremia, with these organisms. It is sometimes hard to separate the roles of foreign bodies versus immunocompromised status. For example, intravascular catheters are an important portal of entry in immunocompromised hosts; however, overgrowth at respiratory and gastrointestinal sites may also serve as sites of entry in such hosts even without a foreign body.

6. Mechanisms and Routes of Transmission

Contact transmission has also been observed with CA-MRSA among athletes (1), prison inmates (2), and day care attendants (3). Transmission of *S. aureus* outside of the hospital occurs through person-to-person spread within families or intimate contacts.[2,23] Studies within the hospital show that the nasal carrier is predisposed to postoperative infection with the same strain.[117] Also within hospitals, other vectors for infection exist with infected patients,[70] health care personnel[75,76] who are infected or are carriers, and fomite contamination.[118,119] Airborne spread of *S. aureus* can be demonstrated[120,121]; however, it has been difficult to correlate counts of staphylococci in air with infection rates. Patients on broad-spectrum antibiotics have been shown to have increased shedding of resistant staphylococci.[122] The infected lesion sheds heavy concentrations of organisms and is potentially the greatest hazard for spreading infection.[123]

Humans are the natural reservoir for *S. epidermidis*; these bacteria are part of the normal skin flora and are the most common staphylococcal species isolated from cutaneous sites. The organism is easily shed from skin and can contaminate inanimate environmental surfaces, other people, and the air. The resistance of the organism to drying and temperature changes produces a long viability.[124] *S. epidermidis* infections result from contamination of a site (usually surgical) by organisms from the patient's skin or nasopharynx or from exogenous sources. Outbreaks of *S. epidermidis* in post-surgical patients have been widely reported.[78,79,83]

7. Pathogenesis and Immunity

7.1. Pathogenesis

Foreign bodies enhance the likelihood that staphylococcal infection will develop by reducing the number of organisms needed to initiate infection. Healthy persons require more than 10^6 virulent staphylococci to cause a minor infection, whereas 10^2 organisms can result in an infection when a foreign body is present.[125] Sutures,[102] intravascular catheters,[110] prosthetic joint devices,[112] artificial heart valves,[126] and tracheostomy[127] and endotracheal[128] tubes are examples of commonly used foreign bodies that may become infected. Fibrin may be responsible for staphylococcal adherence to intravascular catheters.[129] Bacteremia may arise from the local site and can lead to *S. aureus* infection and abscess formation in any organ of the body. Sequelae include such entities as meningitis,[130] osteomyelitis,[131] and endocarditis.[132] Damaged tissue, e.g., the skin in burns and the airways in influenza, are predisposed to colonization and then proliferation of *S. aureus*. A consequence in burn patients is septicemia[133] and in influenza patients, *S. aureus* pneumonia.[105]

S. aureus is a complex organism with a variety of bacterial products with the potential for promoting disease.[134] Potential virulence factors include surface proteins that promote adherence to damaged tissue,[135] bind proteins in blood to help evade antibody-mediated immune responses[135] and promote iron uptake.[136] The organism also expresses a number of proteins that can cause tissue damage by enzymatic action, and superantigen toxins which are potent cytokine inducers.[137] Extracellular products associated with disease include enterotoxins, which produce food poisoning, and exfoliatin which causes scalded-skin syndrome. In toxic shock syndrome, an extracellular toxin (now known as TSST-1) of *S. aureus* related to phage group I is believed to mediate the multiple observed clinical manifestations.[138] Genes that encode virulence factors can be located on mobile genetic elements called pathogenicity islands (e.g., toxic shock syndrome toxin-1 and some enterotoxins),[139] on lysogenic bacteriophages (e.g., Panton–Valentine leucocidin [PVL]),[139,140] or can be integrated into the bacterial chromosome (factors associated with suppressing innate immunity such as the chemotaxis inhibitory protein and staphylokinase).[141]

As with *S. aureus*, the presence of foreign bodies markedly enhances the likelihood of infection with coagulase-negative staphylococci. Indeed, *S. epidermidis* is generally not a pathogen except in the presence of a foreign body, in an immunocompromised host, or in patients with neutropenia. As described earlier, the ability of a coagulase-negative strain of staphylococcus to secrete a slime layer appears to offer it advantages in terms of colonization, persistence, and infection. In one study, 81% of 59 infectious episodes in patients with a prosthetic device were due to a slime-positive coagulase-negative staphylococcus. Slime-positive organisms producing infection were significantly harder to eradicate than slime-negative organisms.[142]

7.2. Immunity

Humans enjoy a high degree of natural resistance to *S. aureus*. The exact nature of this natural immunity is not clear and may be nonspecific.[143] While humans show little resistance to superficial colonization with *S. aureus*, there is great resistance to progressive forms of disease.[143] Spread of staphylococcal infection is limited by reticuloendothelial system clearance and polymorphonuclear leukocyte chemotaxis and ingestion of bacteria.[144,145] This requires blocking the coordinated action of the various cell wall-associated and secreted bacterial proteins.[146] Although IgG

antibodies to specific components of *S. aureus* have been demonstrated,[147] and components of the classic and alternative complement systems have been shown to be necessary at times for efficient phagocytosis of staphylococci,[148] the overall significance of opsonization in immunity to *S. aureus* is unclear.[144,149] IgE antibodies to *S. aureus*, demonstrated in patients with recurrent "cold" abscesses, may represent an abnormal immunologic response to *S. aureus* and more specifically to the cell walls of the bacteria.[150,151]

The role of cell-mediated immunity to *S. aureus* remains to be defined.[149,152] Filtrates from strains of *S. aureus* contain mitogens for human lymphocytes.[153] Delayed hypersensitivity reactions to *S. aureus* have been demonstrated in animals, but are not associated with increased resistance.[152] Indeed, repeated skin infections in a rabbit model produced increased susceptibility to *S. aureus* skin and joint infections, suggesting that delayed hypersensitivity may have a pathogenetic role in staphylococcal disease.[154]

The capsular polysaccharides of *S. aureus* inhibit opsonization and phagocytic killing by polymorphonuclear neutrophils, thereby facilitating the development of invasive staphylococcal infection.[155] Antibodies to the capsular polysaccharides of *S. aureus* types 5 and 8 induce type-specific killing by human neutrophils in vitro and confer protection in animals.[156] When conjugated with carrier proteins, the immunogenicity of the capsular polysaccharides is enhanced by conjugation with carrier proteins.[157–159] A monovalent vaccine containing *S. aureus* type 5 or type 8 capsular polysaccharide bound to recombinant exoprotein A, a nontoxic variant of *Pseudomonas aeruginosa* exotoxin A expressed in *Escherichia coli*, has been developed and was found to be immunogenic and well tolerated.[160]

8. Patterns of Host Response

Staphylococci may infect and cause disease in virtually every organ system of the body. The organism is highly virulent for humans and, with the exception of the anterior nares, the pharynx, and skin, rarely lives in symbiosis with the host. Within the nose and pharynx and on the skin, staphylococci may exist as part of the "carrier" state; their importance in such locations is the capacity to be spread to other sites within the host or to other susceptible individuals. The manifestations of staphylococci infection vary depending on the site involved and will be described separately. Table 1 summarizes some of the features of selected forms of *S. aureus* infections.

Table 1. Clinical Forms of Staphylococcal Infections

Category	Age most often afflicted	Special risk factors
Boils, furuncles, carbuncles	All ages	Diabetes mellitus
Wound infections	All ages	Diabetes mellitus, steroid therapy, obesity, malnutrition, prolonged surgery, foreign body (e.g., prosthesis)
Breast infections	Newborn, women in puerperium	None
Pneumonia	All ages	Drug addiction, influenza outbreaks
Endocarditis	All ages	Heroin addiction, prosthetic heart valve
Skin infections		
Bullous impetigo	Childhood	None
Scalded-skin syndrome	Childhood	Compromised host
Thrombophlebitis	All ages	Intravenous catheters
Bacteremia	All ages	Diabetes mellitus, leukemia, lymphoma
Osteomyelitis	Children	None
Septic arthritis	All ages	None
Food poisoning	All ages	None
Parotitis	Elderly	Dehydration
Toxic shock syndrome	Younger women	Use of vaginal tampons during menses, after childbirth, surgical wounds, influenza
Peritonitis	All ages	Continuous peritoneal dialysis
Urinary tract infection	Young women	None or urinary tract abnormality
Cerebrospinal fluid shunt infection	All ages	Presence of shunt
Pyomyositis	Young men and boys	Tropical climate, HIV
Meningitis	Newborn infants, all ages	Prematurity, endocarditis, CNS disorders

8.1. Clinical Features

8.1.1. Boils, Furuncles, and Carbuncles. A boil or furuncle is an acute abscess of the skin and subcutaneous tissues. Such lesions occur most commonly on the face, neck, buttocks, thighs, perineum, or axillae. Exquisite tenderness is the hallmark of these lesions, which swell with pus and eventually drain spontaneously. Carbuncles are collections of interconnected furuncles that are less likely to drain externally. Carbuncles become larger, are more painful, and are associated with systemic signs and bacteremia; they appear more commonly in association with diabetes.

8.1.2. Wound Infections. *S. aureus* accounts for 17% and coagulase-negative staphylococci account for 12% of surgical wound infections.[161] Factors that have been associated with an increased incidence of wound infection are age, diabetes, steroid administration, obesity, malnutrition, increased duration of time in the operating room, and presence of other infected sites. Wound infections are usually associated with frank purulent drainage, breakdown of the wound, fever, and lack of well-being of the patient.

8.1.3. Breast Infections. Mastitis due to staphylococci generally occurs in newborns and in women (frequently in the puerperium). In neonates, the breast shows marked redness with induration resembling a furuncle. In adults, infection can be superficial, presenting only with pain and a lump; but more extensive involvement may occur with marked tenderness, fever, and leukocytosis.

8.1.4. Pneumonia. Staphylococcal pneumonia may present in one of three ways: lobar pneumonia,[56] diffuse interstitial pneumonia,[154] and localized areas of pneumonia secondary to septic emboli with infarction. In lobar pneumonia, the patients are usually acutely ill with high fever, tachypnea, and cough productive of yellow, bloody sputum. On smear of the sputum, large numbers of neutrophils and staphylococci are seen. Empyema is a frequent occurrence and in infants the development of pneumatoceles is a hallmark of staphylococcal pneumonia. Diffuse interstitial pneumonia usually occurs after or coincident with influenza. The most prominent clinical feature is tachypnea and cyanosis with refractory hypoxia. Localized areas of pneumonia secondary to septic emboli can occur with bacteremia alone or in the presence of endocarditis, particularly involving the tricuspid valve. Pleuritic chest pain, dyspnea, fever, and chills are prominent, whereas cough and purulent sputum are less likely. Roentgenographic examination frequently reveals multiple small densities at the periphery of the lung that frequently increase in size and cavitate.

8.1.5. Endocarditis. Endocarditis complicates the course of *S. aureus* bacteremia in up to 32% of cases, often with devastating sequelae.[162] *S. aureus* was the most common cause of native valve endocarditis in a large cohort of patients.[163] Endocarditis caused by *S. aureus* usually presents as an acute fulminant illness with high fever and metastatic abscesses and has the potential for lethal damage to heart valves. Staphylococcal endocarditis should be suspected in any patient with staphylococcal bacteremia and particularly in heroin addicts. Many of the classic features of endocarditis may be absent and staphylococcal disease frequently involves normal heart valves.[163] Although relatively rare, prosthetic valve endocarditis due to *S. aureus* is associated with high complication and mortality rates.[164]

Only 5% of all cases of infective endocarditis on native cardiac valves are caused by coagulase-negative staphylococci.[165] The disease is subacute and resembles that caused by viridans streptococci. On the other hand, coagulase-negative staphylococci are the most common cause of prosthetic valve endocarditis, accounting for approximately one-third of cases within the first year and only about 10% of later cases.[166] The infections are usually severe with evidence of prosthetic valve destruction and dysfunction in up to 70% of cases.[167] It is presumed that cases caused by coagulase-negative staphylococci occurring in the year following surgery are probably the result of inoculation of organisms at the time of surgery. Diagnosis of coagulase-negative prosthetic valve endocarditis requires a high level of suspicion and the findings of fever and blood cultures positive for these organisms.

More recently, health care-associated endocarditis has become increasingly common among patients with native valve endocarditis due to both *S. aureus* as well as coagulase-negative staphylococci. This subset of endocarditis is characterized by the absence of classical stigmata of endocarditis, predominance of mitral valve involvement, and high mortality rate.[162,168]

8.1.6. Skin Infections. Staphylococci of phage group II have been associated with several manifestations of infection in the skin.[169] These include bullous impetigo, a nonstreptococcal scarlatiniform rash, and toxic epidermal necrolysis (the scalded-skin syndrome). Infected individuals show an abrupt onset with diffuse erythema over most of the body. The skin is exquisitely sensitive to touch and within 1–2 days begins to peel off after light stroking (Nikolsky's sign). Large bullae filled with clear sterile fluid appear in the epidermis, which separates in sheets. Secondary desquamation continues 7–10 days after the onset of redness; recovery is complete.

8.1.7. Intravenous Catheter-Related Infections. Staphylococci are the most frequent cause of infections related to intravenous catheterization. Coagulase-negative staphylococci are most frequently isolated followed by *S.*

aureus.[170] The site of catheter insertion shows signs of local inflammation in only a minority of cases. Patients may present with fever and other systemic signs of sepsis without any obvious source of infection. Blood cultures are usually positive and culture of the intravascular portion of the catheter also yields the infecting organism. Risk factors include the long periods of time that intravenous lines now stay in place; the pressure of multiple antibiotic usages in sick patients, enhancing the likelihood of colonization with multiply-resistant coagulase-negative staphylococci; the ability of the organism to produce a slime layer; the presence of neutropenia; and the administration of other immunosuppressive drugs.

8.1.8. Bacteremia. Staphylococcal bacteremia may arise from any site where staphylococci produce infection. Forty percent of bacteremic patients have no apparent primary site of infection.[171] Bacteremia due to *S. aureus* is now classified into three categories: hospital acquired, community acquired and health care associated.[172] The incidence rates for all of the above categories have significantly increased over the last decade.[173] Hospital procedures such as surgery with subsequent wound infection or parenteral infusion via intravenous catheters are a frequent source. Patients who develop staphylococcal bacteremia frequently have underlying diseases such as diabetes, chronic renal failure, malignancy, or substance abuse.[171] The clinical picture of staphylococcal bacteremia is generally a fulminant illness with metastatic infections occurring in about a third of patients. Shock, disseminated intravascular coagulation, and respiratory failure may occur. There has also seen a significant rise in rates of MRSA recently. Approximately 30% of *S. aureus* bacteremia isolates in the United States are now methicillin resistant.[174] Widespread antibiotic use and poor adherence to infection control precautions have both contributed to the rise in MRSA infection rates.[175]

Coagulase-negative staphylococci are now the most frequent cause of hospital-acquired bacteremia.[161] The recent increase in incidence is related to the increase in the number of immunocompromised patients and an increase in the use of centrally placed intravenous catheters. Neutropenic patients are at particular risk, and the mortality in these patients is as high as 34%.[176,177]

8.1.9. Osteomyelitis. *S. aureus* is the most common cause of acute osteomyelitis.[178] It occurs most frequently in children under 12 years of age but also may be seen in adults. The clinical picture begins abruptly with fever, chills, and pain at the site of involvement. The skin overlying the bone is often warm, red, and swollen. Cultures of bone or pus will generally reveal staphylococci, and bacteremia is present in about one half of the patients. Long bones are most commonly affected in children, while vertebrae are a more common site in adults. The clinical picture of vertebral osteomyelitis may be only that of malaise and vague back pain, and an elevated erythrocyte sedimentation rate may be the only laboratory abnormality. With progression, painful muscle spasms, limitation of motion of the spine, and low-grade fever will usually develop. Osteomyelitis can occur following traumatic injury, bone and joint surgery, or as a complication of staphylococcal bacteremia. Chronic staphylococcal osteomyelitis may occur if therapy of the acute disease is delayed or even in some instances following proper treatment. A classical example is a diabetic foot ulcer infection with chronic osteomyelitis. The characteristic clinical course is an indolent one in which sinuses drain purulent material for many years.

8.1.10. Septic Arthritis. Septic arthritis in all age groups is most frequently caused by *S. aureus*.[179] The typical presentation occurs with fever, pain, and chills, and the diagnosis rests on aspiration, Gram stain, and culture of the joint fluid. Staphylococci are the most frequent cause of infected joint prostheses; *S. aureus* and coagulase-negative staphylococci are isolated with approximately equal frequency.[180] It is presumed that infections with coagulase-negative staphylococci are a result of operative contamination; however, there may be a prolonged latent period before clinical infection occurs and it is not possible to exclude hematogenous dissemination as a mechanism. The symptoms of infection may be mild, consisting only of pain in the area of the prosthesis and fever. The diagnosis must be made by culture of the joint space. Although such infections are not common, they are devastating complications because removal of the prosthesis is usually required.

8.1.11. Food Poisoning. Staphylococcal food poisoning is produced by the ingestion of food containing enterotoxin produced by *S. aureus*. The usual interval between ingestion of food containing the toxin and the onset of symptoms is relatively short, ranging from 30 min to 7 hr. Onset is usually abrupt, with severe nausea and cramps followed by vomiting, diarrhea, and prostration. Fever is usually absent and the duration of illness is generally less than 24 hr. The short incubation period, brief duration of illness, and absence of fever help distinguish staphylococcal food poisoning from other forms of food poisoning.

8.1.12. Parotitis. *S. aureus* has been recognized as one of the most common causes of acute suppurative parotitis.[179] The disease typically occurs in elderly postoperative patients who are dehydrated or intubated.[181,182] Other predisposing factors include recent intensive teeth cleaning, use of anticholinergic drugs, and salivary calculi with obstruction. Clinically, the parotid gland is erythematous, swollen, and very tender. High fevers, chills, and elevated white cell counts are generally present. Suppura-

tive parotitis also has been reported in neonates[183] and children,[184] with *S. aureus* isolated from parotid pus.

8.1.13. Toxic Shock Syndrome. This entity has been described predominately in young women who are actively menstruating, but it can also appear following all types of surgery, after childbirth, with wounds, in children, or after influenza. The individuals develop fever, an erythematous macular rash that subsequently desquamates, falling blood pressure, and then multisystem involvement. The latter has included gastrointestinal, renal, hepatic, hematologic, central nervous, and cardiopulmonary systems. *S. aureus* has been found in the vaginal cultures of nearly all menstrual cases and has been recovered from wounds in other cases. A significantly higher proportion of menstruating women with this syndrome used vaginal tampons when compared with controls.[35,36] Other diseases must be excluded, such as meningococcemia, Rocky Mountain spotted fever, or bacteremia. Toxic shock syndrome has also been reported to be caused by strains of coagulase-negative staphylococci that produce TSST-1.[37] In such cases, coagulase-negative staphylococci that produce this toxin were isolated from the vagina and no coagulase-positive staphylococci were recovered.

8.1.14. Peritonitis. Chronic ambulatory peritoneal dialysis is an attractive alternative to hemodialysis for patients with chronic renal failure. One complication, however, is the development of peritonitis. Coagulase-negative staphylococci are the predominant causative agents although their relative rates are decreasing.[185,186] Because the number of organisms in the peritoneum may be small, inoculation of larger volumes of peritoneal fluid and filtration of large volumes of fluid followed by culture of the filter have been tried to enhance the likelihood of diagnosis. Treatment of these infections is generally successful without catheter removal, either with systemic therapy or with intraperitoneal administration of antibiotics.[187]

8.1.15. Urinary Tract Infection. Although not commonly thought of as a urinary tract pathogen, coagulase-negative staphylococcus (*S. saprophyticus*) is the second-most common cause of acute urinary tract infections in young women.[188] The organism is cultured infrequently from the genitourinary tract of young women, but there is a high correlation between colonization and subsequent development of urinary tract infection. Identification of the organism is facilitated in the clinical microbiology laboratory because of its resistance to novobiocin. The organism causes both upper and lower urinary tract disease, and the signs, symptoms, and examination of the urine of women infected with this organism are indistinguishable from those of patients infected with gram-negative enteric organisms. Other coagulase-negative staphylococci do not generally infect the urine, but do account for some infections in older patients who are usually hospitalized and have underlying urinary tract complications (e.g., indwelling urinary catheter, recent urinary surgery, bladder dysfunction, or obstruction).[189] These latter infections with coagulase-negative staphylococci account for less than 5% of all episodes of significant bacteriuria in hospitalized patients. The isolation of *S. aureus* from the urine occurs almost exclusively as a complication of *S. aureus* bacteremia and hematogenous spread to the kidney rather than as an ascending urinary tract infection.

8.1.16. CSF Shunt Infection. The most common organism causing infection of CSF shunts is coagulase-negative staphylococcus. In one series, close to 50% of the infections were caused by *S. epidermidis*.[190] The diagnosis often depends on the type of the shunt; patients with ventriculoatrial shunts usually have bacteremia, while those with ventriculoperitoneal shunts most often have peritonitis. The signs and symptoms may be only low-grade fever and shunt malfunction. Examination of the spinal fluid is important, but pleocytosis is usually mild. In general, shunt removal is required to cure infection.

8.1.17. Pyomyositis. *S. aureus* can infect a single muscle or less commonly several muscles in healthy young men or boys in tropical climates. Cases have been reported from temperate climates; infection with human immunodeficiency virus is a major risk factor.[191] Fever, muscle pain and swelling, and leukocytosis are common. Bacteremia can occur. Drainage and antibiotic therapy are usually effective.[192]

8.1.18. Meningitis. In published studies, approximately 1–9% of bacterial meningitis is caused by *S. aureus*.[193] Meningitis due to *S. aureus* occurs as a consequence of a neurosurgical procedure or as a hematogenous complication of an overwhelming, disseminated infection such as bacteremia or endocarditis.[194] Mortality in postoperative meningitis is 18%, but exceeds 50% in hematogenous infection.

8.2. Diagnosis

Because of the multiplicity of manifestations, there are no pathognomonic features that allow one to establish a diagnosis of staphylococcal infection immediately. There are, however, certain epidemiological, clinical, and laboratory features that help to establish the diagnosis or to raise suspicion. Outbreaks of nursery infections or postoperative surgical wound infections generally are caused by staphylococci; recovery of the causative organism and identification of an individual who may be responsible for shedding

the organism should be considered promptly. Heroin addicts appear particularly prone to staphylococcal infections and this organism should be considered when such patients present with fever. Other groups of patients such as diabetics, patients with hematologic malignancies, and patients with granulocytopenia appear particularly prone to staphylococcal infections. Skin manifestations of the scalded-skin syndrome may be sufficient to establish a diagnosis of staphylococcal infection. Ultimately, however, with rare exceptions, the definitive diagnosis of staphylococcal infection depends on isolating the organism for visualizing it by Gram stain from pus or other body tissues and fluids.

Epidemiological studies in the past have used phage typing of *S. aureus*. Newer studies have used molecular techniques such as restriction endonuclease analysis of plasmid DNA,[195] pulsed-field gel electrophoresis of DNA,[196] and polymerase chain reaction amplification of select DNA sequences,[197] all with promising results. In addition, there are typing systems for coagulase-negative staphylococci.[29]

9. Control and Prevention

Most *S. aureus* infections occurring in healthy persons living in the community are sporadic events that are caused by organisms carried in the nares and on the skin. Although good hygiene and proper care of minor wounds may reduce the incidence of such infections, more extensive control efforts are unlikely to be of benefit. Immunocompetent persons with recurrent staphylococcal skin infections are likely to be chronic nasal carriers, and multiple family members may be colonized. Eradication of the carrier state with agents such as intranasal mupirocin or intranasal mupirocin may decrease the frequency of infection. Patients receiving hemodialysis or peritoneal dialysis who are nasal carriers of *S. aureus* are at increased risk for serious staphylococcal infections. Elimination of the carrier state reduces the risk of infection.[62,198]

Within hospitals and nursing homes, patients colonized or infected with *S. aureus* are the major source of the organism. Chronically colonized health care workers may occasionally be the source of epidemics of staphylococcal infection. More importantly, health care workers spread staphylococci from patient to patient by transient hand carriage. Use of gloves when handling potentially infectious materials and proper handwashing between patient contacts are essential to minimize transmission between patients. More stringent isolation procedures are indicated for patients with active lesions, extensive wounds or burns, or respiratory tract infections. MRSA has been particularly difficult to control in many hospitals. Laboratory-based surveillance for MRSA permits identification of colonized and infected patients so that appropriate isolation measures may be instituted.[46,96]

Attention to details of patient care such as meticulous surgical technique, proper care of vascular catheters, and avoidance of unnecessary antibiotics can reduce the incidence of staphylococcal infection. Administration of prophylactic antibiotics immediately before surgical procedures is justified in procedures in which the risk of infection is high or the consequences of infection are severe.[200]

Treatment of staphylococcal infection with β-lactam antibiotics or vancomycin often fails to eliminate the carrier state. Asymptomatic carriers may continue to serve as reservoirs of the organism. Eradication of MRSA carriage has proved to be problematic as MRSA strains are often resistant to multiple antimicrobial agents and often become resistant to drugs used to eradicate carriage. A variety of topical and systemic agents, including mupirocin, rifampin, trimethoprim sulfamethoxazole, ciprofloxacin, novobiocin, and minocycline, have been used alone and in combination with variable results, and emergence of resistance to the agent or agents has been a recurring problem.[201] A vaccine directed against *S. aureus* capsular polysaccharide has shown short-term efficacy in hemodialysis patients.[202] The possible role of such an approach in the prevention of MRSA infection is not known.

The optimal approach to control of infection with coagulase-negative staphylococci is uncertain. The skin is heavily colonized with these organisms and patients often acquire multiple antibiotic-resistant strains after admission to the hospital.[203] Infection results when normal defenses are disrupted by such medical interventions as surgery, implantation of foreign bodies, and cytotoxic chemotherapy. Innovative approaches to prevent the attachment of staphylococci to the implanted cuff attached to central venous catheters significantly decreases catheter-related infection.[204] New technologic advances in prostheses implantation such as antibiotic beads or antibiotic-impregnated cement spacers are promising and seem to decrease the rates of postoperative infections.[205]

10. Unresolved Problems

Despite many years of intensive work toward control of *S. aureus*, there are numerous areas for continued investigation. Factors related to the host or organism itself, which make for chronic as opposed to transient carriage, remain undefined. We still do not know the rates of endogenous infection among transient compared with persistent carriers outside the hospital setting. Studies of well-defined popula-

tions are needed to assess whether sex differences exist for rates of *S. aureus* disease. With new ways to treat formerly fatal diseases, we need ongoing studies to define additional populations at high risk for serious *S. aureus* disease.

Our understanding of natural and acquired immunity to *S. aureus* is sorely lacking when compared to our understanding of other bacterial pathogens. What is lacking in the "normal" child that allows for disseminated *S. aureus* infection? Conversely, in the "normal adult," why is disseminated disease so infrequent? If mortality from *S. aureus* is to be reduced in the high-risk population already defined, more studies are needed to determine vaccine efficacy in these patients.

Perhaps the most pressing problem is the emergence of *S. aureus* isolates with intermediate and high-level resistance to glycopeptides (1, 2). Different mechanisms account for the two types of resistance. Vancomycin-intermediate *S. aureus* (VISA) harbor mutations that result in thickening of the peptidoglycan layer (3, 4). Such resistance might be overcome with high doses of vancomycin. On the other hand, vancomycin-resistant *S. aureus* (VRSA) have acquired the VanA resistance gene from enterococcal species and therefore do no exhibit a dose-dependent resistance to vancomycin (1, 5). Although vancomycin resistance rates are still low, the emergence of such strains might be inevitable, especially with the persistent intense glycopeptide use. Resistance to newer anti-staphylococcal agents like daptomycin has already been reported.

A major problem with *S. epidermidis* is determining whether the organism, when isolated from blood, is a true pathogen or a contaminant. If one looks at all patients in hospitals, only about 5–10% of individuals having one or more blood cultures positive for coagulase-negative staphylococci have a "true" clinically significant episode of bacteremia.(211) In contrast, in patients who are immunocompromised or have malignancies with indwelling intravenous catheters, the likelihood that a blood culture growing *S. epidermidis* represents significant clinical infection is substantially higher. The availability of sensitive, specific, inexpensive, and easily performed typing systems for coagulase-negative staphylococci could be of help to the clinical microbiology laboratory, to clinicians, and to infection control personnel trying to contain the spread of nosocomial infection caused by these organisms.

A second major problem with *S. epidermidis* is determining optimal antimicrobial therapy. Many of these organisms are resistant to multiple different antibiotics, and often the clinician is left with only vancomycin to use as treatment. The precise role of the addition of rifampin is not clear, and further controlled studies are needed to determine whether addition of this agent truly improves the outcome of infection with *S. epidermidis*.

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CHAPTER 35

Streptococcal Infections

Barry M. Gray and Dennis L. Stevens

1. Introduction

The streptococci are a large heterogeneous group of gram-positive spherically shaped bacteria widely distributed in nature. They include some of the most important agents of human disease as well as members of the normal human flora. Some streptococci have been associated mainly with disease in animals, while others have been domesticated and used for the culture of buttermilk, yogurt, and certain cheeses. Those known to cause human disease comprise two broad categories: First are the pyogenic streptococci, including the familiar β-hemolytic streptococci and the pneumococcus. These organisms are not generally part of the normal flora but cause acute, often severe, infections in normal hosts. Second are the more diverse enteric and oral streptococci, which are nearly always part of the normal flora and which are more frequently associated with opportunistic infections.

Measured in terms of mortality, morbidity, and economic costs, five streptococcal species are of major importance in human disease. (1) The group A *Streptococcus*, *Streptococcus pyogenes,* produces a wide range of infections, from pharyngitis and impetigo to puerperal sepsis, erysipelas, necrotizing fasciitis, and toxic shock syndrome. Their nonsuppurative sequelae include acute rheumatic fever and acute glomerulonephritis. (2) The group B *Streptococcus*, *Streptococcus agalactiae,* is currently a leading cause of sepsis in newborn infants and a frequent cause of postpartum infections in mothers. (3) The pneumococcus, *Streptococcus pneumoniae,* remains the most frequent cause of bacterial pneumonia in all age groups and is a common agent in otitis media, bacteremia, and meningitis. A detailed account of the pneumococcus may be found in Chapter 29. (4) Among the oral streptococci, *Streptococcus mutans* is important as a principal agent of dental caries. (5) The *Enterococcus* is part of the normal bowel flora and has been increasingly isolated as an opportunistic invader, especially in nosocomial infections. *Enterococcus* is now a separate genus but will be considered an honorary *Streptococcus* in this chapter because of its similarities to enteric streptococci.

2. Historical Background

Streptococcal infections were recognized by Greek physicians by the third century BC. A description of erysipelas is recorded in *Epidemicus* and attributed to Hippocrates. In the Middle Ages, scarlet fever, or "scarlatina," as it was called in Italy, was an eye-catching and notable disease. Sydenham's description in 1676 clearly differentiated this disease from measles and other rashes, but it was not until 1924 that G.F. and G.H. Dick showed conclusively that streptococci were the causative agents. Until the advent of penicillin, childbed fever, or puerperal sepsis, remained one of the most frequent causes of death among otherwise healthy young women. The classic works of Holmes, in 1858, and Semmelweiss, in 1861, described the transmission of this disease and provided guidelines for effective infection control and preventive measures that are still applicable today. Rheumatic fever was first described by Wells in 1812, and Bouillaud described the association of acute rheumatism and heart disease in 1835. In 1836, Bright published his account of "renal disease accompanied with secretion of albuminous urine."[1] Osler provided detailed descriptions of "malignant scarlet fever," which remained common until the advent of antibiotics (see Figure 1). Severe invasive diseases, including necrotizing fasciitis, "hemolytic streptococcus gangrene," and myositis were much less common and did not appear in the medical literature until the second and third decades of this century. During the mid-1970s to 1980s,

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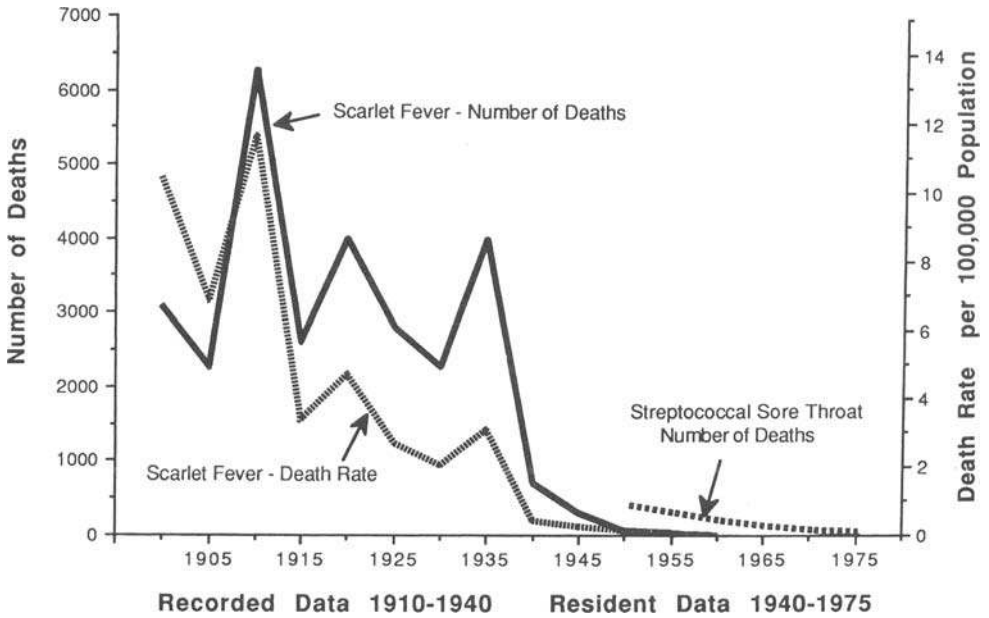


Figure 1. The number of deaths (*left-hand scale*) from scarlet fever (1900–1960) and from all streptococcal sore throat (1950–1975), with death rates (*right-hand scale*) per 100,000 population in the United States. Appropriate comparability ratios were applied beginning in 1940, and recorded data compiled according to the geographic locale where the event occurred without regard to residence (1900–1940); resident data were compiled according to the usual place of residence without regard to locale where the event occurred (1940–1975). The population used for determining rates was that of the registration area. Data were not available for streptococcal sore throat for 1900–1950. The number of states reporting for 1900–1905 was 10, gradually increasing to 48 in 1935 and to 50 states in 1960. After 1975, reporting was optional, and no accurate data are available. Sources of data: US Bureau of the Census,[234] National Office of Vital Statistics,[235] Centers for Disease Control.[236] Figure redrawn from Quinn.[1]

cases of acute rheumatic fever and acute glomerulonephritis became exceedingly rare (see Figure 2), and streptococcal disease seemed to become only an inconvenience. By the late 1980s, however, rheumatic fever made dramatic reappearance at military posts and in Utah and other areas of the United States.[2,3] There was also a dramatic increase in severe invasive infections and the emergence of a streptococcal toxic shock syndrome.[4] Group A streptococci made the headlines in the popular press as "Killer Strep," the "flesh-eating bacteria."

In the late 19th century, manyinvestigators contributed to the understanding of streptococci and their relation to human disease. By the 1880s many species had been given names such as *Streptococcus epidemicus, Streptococcus erysipelatus, Streptococcus scarlatinae,* and *Streptococcus rheumaticus,* which reflected different manifestations of streptococcal infection. The name *S. pyogenes* dates from this period but is probably of less descriptive value than the term "*Streptococcus haemolyticus,*" which was commonly used through the early part of this century. The formal classification of streptococci began when blood agar came into use and the hemolytic properties of various organisms were noted. In 1919, Brown used the term "beta" to describe streptococci that produced a 2- to 4-mm zone of clear hemolysis around colonies grown on blood agar. "Alpha" streptococci were those producing incomplete, greenish, hemolysis. Most of the isolates from severe human disease were β-hemolytic. It was not until 1928, when Lancefield introduced methods of serotyping streptococci based on immunologic reactions with cellular components, that groups and types within groups could be clearly distinguished. The group antigens were eventually shown to be specific cell wall carbohydrates. The group A streptococci were further differentiated by the M and T protein antigens. The β-hemolytic streptococci from most human infections proved to be those of group A. Armed with these new epidemiologic tools, Lancefield and Hare investigated cases of puerperal sepsis at Queen Charlotte's Hospital in London, beginning in the early 1930s.[5] Of 46 cases of postpartum sepsis, all but oneisolate was group A, and the exception was identified as the prototype of a new serologic group, designated group G. A year earlier, Hare and Colebrook observed that hemolytic streptococci resembling those associated with sepsis were never found in vaginal cultures of healthy women, but that some women carried streptococci that resembled those isolated in bovine mastitis. The latter organisms proved to be members of *Streptococcus* group B.[5]

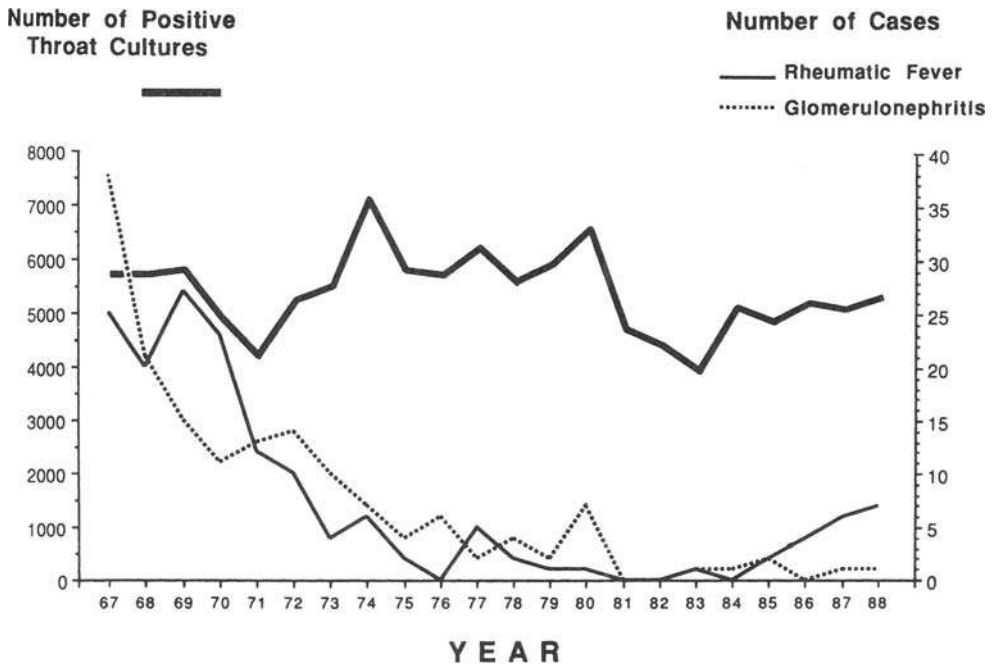


Figure 2. The number of positive throat cultures (*left-hand scale*) and number of confirmed cases of rheumatic fever and acute glomerulonephritis (*right-hand scale*) seen in private pediatric practices participating in streptococcal surveillance studies in Rochester, New York, 1967–1988. The data were kindly provided by Caroline Breese Hall, University of Rochester.

By the late 1930s the group B streptococci were recognized as important, if occasional, pathogens causing postpartum sepsis, amnionitis, endocarditis, and septic abortion. The advent of antibiotics, along with better methods for preventing nosocomial spread, resulted in a dramatic decline in streptococcal infections on obstetrical services. At Queen Charlotte's Hospital there were very few maternal deaths due to either group A or group B streptococci from 1940 to the mid-1960s. In this same period there were few cases of neonatal sepsis or meningitis attributed to streptococci of any kind in large series published in the pediatric literature. It appears unlikely that group B streptococci went simply unrecognized as perinatal pathogens for over 20 years. Rather, there seems to have been a real increase in group B disease, beginning in the United States and Europe during the 1960s. By the mid-1970s, numerous reports of group B disease appeared in the literature, and the group B *Streptococcus* was said to have come of age.

The pneumococcus has an interesting and important history, beginning with its association with pneumonia in the 1880s and later with developments in immunology, antibiotics, and the discovery of DNA.[6]

The history of *S. mutans* began in 1924, when Clark first isolated the organism from human dental caries. It was another four decades, however, before Keyes established the "infectious and transmissible nature" of dental caries in animal models and later in human subjects.[7–9] Since dental caries are chronic rather than acute infections and are not directly life-threatening, the global significance of *S. mutans* has only recently been appreciated. Dental caries, a complex but diagnosable and treatable infection, is perhaps the most common bacterial infection in humans.

Streptococcus suis is an emerging zoonotic microbe contracted by humans having direct contact with pigs and pork products. The first human infections were reported in a series of meningitis cases beginning in 1968.[10] Over the past decade several large outbreaks and over 400 human cases have been reported mainly from Asia and Europe.[11]

The enterococci, as they were called by Thiercelin in 1899, were noted as a major aerobic component of feces. Extremely hardy, they grow in bile, at high or low temperatures, in salt, and at high pH. Although for many years grouped with the streptococci because of morphologic similarities, they were transferred to the genus *Enterococcus* in 1984 on the basis of genetic studies. These and several new *Enterococcus* species were clearly distinct from the "nonenterococcal" group D streptococci, *Streptococcus bovis* and *Streptococcus equinus.* Enterococci have long been known as an occasional cause of opportunistic infections, but in the early 1970s they began to appear with increasing frequency in reports of urinary tract infection, bacteremia, and

pelvic, wound, and surgical infections. Vancomycin-resistant enterococci (VRE) are now among the most common organisms causing nosocomial infections and are difficult to eradicate because of resistance to an increasing number of antibiotics.[12]

3. Methodology

3.1. Sources of Mortality Data

Until the last decade there have been few reliable sources of mortality data for any of the streptococcal diseases. Cause-of-death coding classifications have been revised about every decade, usually without sufficient details regarding a particular etiologic agent. For example, patients may die of heart failure without rheumatic heart disease being specified; puerperal sepsis may be due to group A or group B streptococci as well as other organisms; pneumococcal pneumonia deaths are frequently counted with those attributed to influenza virus.

3.2. Sources of Morbidity Data

The most reliable data on morbidity from streptococcal diseases focus on severe invasive infections from national and regional survey areas in the United States, Canada, Europe, and Australia. The Centers for Disease Control and Prevention (CDC) began a passive nationwide surveillance for invasive group A streptococcal infections in 1993. This was expanded to include 10 large metropolitan and regional areas (about 30 million persons or 10% of the US population) as part of the Active Bacterial Core surveillance (ABCs) program of the Emerging Infections Programs Network.[13] Surveillance of group B streptococcal disease was subsequently added.[14] The ABCs have also facilitated tracking of pneumococcal disease incidence and of drug-resistant pneumococci.[15] In Europe, until recently, an informal questionnaire system has been used to track group B streptococcal disease rates[16] and pneumococcal disease has been assessed mainly by review of published reports from individual countries and localities. These efforts have now evolved into more integrated international programs, including strep-EURO[17] and Pcn-EURO.[18] Some national public health centers publish their own more detailed surveillance data. Of particular interest are reports on invasive groups A, B, C, and G streptococcal infections in Denmark 1999–2002[19] and a 10-year survey of invasive group A streptococcal infections in the Netherlands.[20] There has been little monitoring of enterococcal infections and vancomycin-resistant enterococci except in the United States from reports of hospitals participating in the National Nosocomial Infections Surveillance System.[21]

There are few accurate data on morbidity from noninvasive streptococcal infections. Since 1971, streptococcal sore throat, scarlet fever, and acute rheumatic fever have been reported only optionally to the CDC, which now relies primarily on selected hospital and population surveys. Monitoring of recent outbreaks of rheumatic fever by the CDC has been incomplete, and no mechanism for systematic nationwide reporting has been adopted. The best longitudinal incidence data on rheumatic fever are from Denmark, where meticulous records have been kept for over a 100 years.[22] The global burden of severe disease, including acute rheumatic fever, rheumatic heart disease, acute glomerulonephritis, and invasive disease is at least 18 million cases, with about 1.8 million new cases per year.[23]

3.3. Surveys

A considerable number of surveys have been published on diseases caused by various streptococcal species. The most informative have been prospective epidemiologic studies, certain family studies, and surveys of defined populations. These will be reviewed in the description of epidemiologic features of specific diseases in Section 5.1.

3.4. Laboratory Diagnosis

The major streptococcal species associated with human disease are listed in Tables 1 and 2, along with selected biological and clinical features. Our grouping of species is more for convenience than for taxonomic purposes, and various bacteriology textbooks will have different schemes, as well as more detailed information on laboratory methods. Recent changes in taxonomy and nomenclature have been reviewed by Facklam[24] and Kohler.[25] The laboratory procedures and the particular approach to identification will of course depend upon whether isolation is for clinical or research purposes and the degree of specificity required.

3.4.1. Isolation and Identification.

3.4.1.1. Media and Growth Conditions. Most streptococci grow poorly on ordinary nutrient media but grow quite well on media enriched with blood, brain, or heart infusion, serum, or glucose. Todd–Hewitt broth is an enriched buffered medium that has become a standard liquid medium for most streptococci. In the United States most laboratories use 5% sheep blood agar, whereas in Britain horse blood is generally used. Both give good hemolysis, although with sheep blood it may be necessary to "stab" the agar in the area of inoculation to ensure subsurface growth for the action of oxygen-sensitive hemolysins. While most streptococci grow well in a

Table 1. The Major Species of Pyogenic Streptococci Causing Disease in Humans

	Species designation	Lancefield group	Hemolysis	Presumptive identification	Definitive identification	Ecological niche	Normal flora	Association with disease
Group A. streptococci	*S. pyogenes*	A	β	Bacitracin sensitivity, latex agglutination, and other antigen detection methods	M proteins T proteins Opacity factor Molecular methods	Oropharynx	No (modest carrier rates)	Pharyngitis, impetigo, erysipelas, bacteremia
Group B streptococci	*S. agalactiae*	B	β	CAMP test positive	Capsular polysaccharides	Lower bowel, genital tract	High carrier rate in normal population	Neonatal sepsis, meningitis, postpartum infections, endocarditis, cellulitis
Group C streptococci	*S. equisimilis*	C	β	"Not A or B"	c-Protein DNA fingerprint DNA fingerprint	(Swine, humans)	Occasional	Pharyngitis, bacteremia, wound infections, cellulitis, often veterinary related
	S. zooepidemicus	C	β	Latex agglutination	Bacteriophage	(Horses, cattle, other animals)	No	
	S. equi	C	β				No	
Group G streptococci	"Large-colony" group G	G	β	"Not A or B" Latex agglutination	Some with M-12 protein	Pharynx, bowel	Occasional	Pharyngitis, cellulitis, bacteremia
Pneumococci	*S. pneumoniae*	None (has pneumococcal C-polysaccharide)	α	Optochin sensitive, bile soluble	Capsular polysaccharides Surface protein Molecular methods	Nasopharynx	High carrier rate in normal population	Lobar pneumonia, otitis media, bacteremia meningitis

Table 2. The Major Species of Oral and Enteric Streptococci and Enterococci Causing Disease in Humans

	Species name	Lancefield group	Hemolysis	Presumptive identification	Definitive identification	Ecological niche	Normal flora	Association with disease
Dental/oral and "Viridans" streptococci	*S. mutans*	None, E	None	—	Cell wall carbohydrate DNA hybridization	Tooth surface, dental plaque	Yes	Dental caries, endocarditis
	S. sanguis	None, H	α, β, none	—	Biochemical	Dental plaque	Yes	Endocarditis, brain abscesses, bacteremia
	S. mitis	None, O, K, M	α, β, none	—	Biochemical	Oral cavity	Yes	
	S. salivarius	None, K	None	—	Biochemical	Tongue	Yes	Bacteremia
	S. sobrinus		α, none	—	Biochemical	Oral cavity	Yes	Bacteremia
	S. oralis		α, none	—	Biochemical	Oral cavity	Yes	Bacteremia
S. anginosis group ("Milleri")	Minute colony	A, C, F, G, none	α, β, none	—	Biochemical	Oral cavity	Yes	Endocarditis, dental abscesses, brain abscesses, liver abscesses
	S. anginosis	C	β	—	Biochemical	Oral cavity	Yes	
	S. costellatus			—	Biochemical	Oral cavity	Yes	
	S. intermedius			—	Biochemical	Oral cavity	Yes	
Nutritionally deficient streptococci	*S. mitis* (mutant)	Various	α, β, none	Require pyridoxal	Biochemical	Oral cavity	Yes	Endocarditis
	S. sanguis (mutant)	Various	α, β, none	Require pyridoxal	Biochemical	Oral cavity	Yes	Endocarditis
	S. salivarius (mutant)	Various	α, none	Require pyridoxal	Biochemical	Oral cavity	Yes	Endocarditis
Microaerophilic and anaerobic streptococci	*Peptostreptococcus* (five species)	Some D	None	—	Biochemical	Oral cavity, bowel	Yes	Abscesses, otitis media, pelvic inflammatory disease
Nonenterococcal group D	*S. bovis*	D	α	—	Biochemical	Bowel	Yes	Bacteremia, endocarditis, opportunistic infections
	S. equinus	D	α	—	Biochemical	Bowel	Yes	
Enterococci (>20 species)	*E. faecalis*	D	None, β	Growth in bile-esculin 6.5% NaCl	Biochemical	Bowel	Yes	Urinary tract and surgical infections, bacteremia, endocarditis, wounds, opportunistic infections
	E. faecium	D	α, β, none		Biochemical	Bowel	Yes	

normal atmosphere, some species, particularly the "*Streptococcus milleri*" group and the peptostreptococci, do not show good growth or hemolysis without a reduced oxygen atmosphere. Pneumococci prefer to grow in 5% CO_2 or in a candle jar. Most streptococci grow best at 35–37°C, although the enterococci will also grow at 10 and 45°C.

Routine isolation of group A streptococci from throat cultures is adequately accomplished using plain sheep blood agar incubated in air at 37°C; cultures read as negative at 18–24 h should be incubated an additional 24 h for optimal sensitivity. Alternatively, throat cultures may be incubated anaerobically, although this may increase the recovery of nongroup A β-hemolytic streptococci. Selective media containing trimethoprim–sulfamethoxazole have also been evaluated and recommended for use in throat cultures.

Many laboratories now process vaginal or anorectal cultures to determine colonization by group B streptococci in pregnant women during the third trimester. A selective broth medium is generally required to minimize overgrowth of other organisms. A Todd–Hewitt broth containing nalidixic acid, polymyxin, and crystal violet has been used in large epidemiologic studies.[26,27] A similar medium is available with colistin plus nalidixic acid but without crystal violet ("Lim" broth).[28] A broth containing gentamicin plus nalidixic acid has also been widely used, although some group B strains may be inhibited by the gentamicin. A satisfactory commercial solid medium is Columbia CNA agar, which contains colistin and nalidixic acid. New Granada medium has the advantage of revealing the orange-pigmentation characteristic of group B streptococci grown in the presence of starch, but a liquid version of this medium has proved unsatisfactory.[29] For isolation of pneumococci from nasopharyngeal carriers a selective blood agar containing 4 mg/l of gentamicin sulfate, Columbia CNA agar, and plain blood agar are usually satisfactory. The enterococci grow on nearly all conventional media, including EMB (eosin–methylene blue) used for gram-negative enteric organisms.

3.4.1.2. Colony Morphology and Hemolysis. The first step in identifying a *Streptococcus* is examining colonies grown on blood agar for characteristic morphology and hemolysis. A throat culture, for example, will have much extraneous growth, but the bacteriologist will be looking for group A streptococci that have small (0.5–1 mm) opaque white colonies surrounded by a wide zone of clear β-hemolysis. Stabs made into the medium in the area of inoculation will usually clear first because the streptolysin O is more active in the absence of air. Streptolysin S, which is active on the surface, does not always produce complete hemolysis in young cultures. Occasional strains produce large (>1 mm) mucoid colonies, due to the expression of hyaluronic acid capsules (especially M type 18).

Groups A, C, and G streptococci are similar in appearance, but group B streptococci are usually larger, more mucoid, and have a narrower zone of hemolysis that is often less distinct. About 1% of wild group B strains are nonhemolytic, but this depends to some degree on the media used. Some streptococci of the *Streptococcus anginosis* ("*milleri*") group have very tiny colonies but comparatively large zones of β-hemolysis; these may include group F and the "minute colony" groups A, C, and G streptococci.[24] The α-hemolytic streptococci are quite heterogeneous and are difficult to distinguish even to the practiced eye. The pneumococci, however, are usually smooth and glossy with central craters formed by autolysis as the colonies grow beyond 18–24 h. Colonies of type 3 pneumococci are often very large and have a distinctive mucoid appearance. Enterococci have rather buttery colonies, compared to most of the streptococci, and usually produce no hemolysis or may be β-hemolytic on horse blood and α-hemolytic on sheep blood.

3.4.1.3. Presumptive Identification. For practical purposes, group A streptococci can be identified presumptively by the bacitracin sensitivity test of Maxted in which a 0.04-unit bacitracin (TAXO^R) disk is placed on a pure subculture of a β-hemolytic *Streptococcus*. Group A streptococci are extremely sensitive and uniformly show a zone of inhibition, whereas only 5–10% of group C or G strains may give a false-positive result. The simplest of several tests for group B streptococci is the CAMP test (described by Christie, Atkins, and Munch-Petersen in 1944). The test is performed by inoculating a streak of the group B streptococcus perpendicular to a streak of *S. aureus* on a sheep blood agar plate; an arrowhead-shaped area of complete hemolysis indicates the presence of CAMP factor, which enhances the effect of the staphylococcal β lysin. Streptococci reported simply as "not A or B" on the basis of presumptive tests usually prove to be group C or G. Pneumococci are distinguished from other α-hemolytic streptococci by sensitivity to optochin in a manner similar to that of the bacitracin test for group A streptococci. In addition, the bile solubility test of Neufeld remains useful for separating pneumococci from the occasional optochin-sensitive viridans *Streptococcus*. Enterococci are identified by growth on bile-esculin media and in 6.5% NaCl. Further identification of these and the viridans streptococci is done with various panels of biochemical tests.

3.4.1.4. Rapid Antigen Detection Tests (RADT). A number of direct antigen detection tests are currently in use for diagnosis of streptococcal pharyngitis. These RADTs yield results in 10–60 min and permit the physician to appropriately treat streptococcal pharyngitis without the delay of conventional cultures. Older latex or particle agglutination tests have good specificity but poor sensitivity.

Most newer methods are based upon the extraction of cell wall antigens from throat swabs followed by their detection by enzyme immunoassay (EIA). In general, these tests have excellent specificity (>95%), but the sensitivity is on the order of 80–90%, or even lower, compared to culture. Some optical immunoassays and chemiluminescent DNA probe tests may approach the accuracy of culture. However, there are conflicting results among studies and few that directly compare various methods. For this reason it is recommended that a throat culture be done when the rapid test is negative. Individual laboratories should verify their own test parameters to assess the need for back-up cultures and minimize the risk of false-negative results.[30]

Two problems continue to limit the usefulness of the RSDTs. One is that they detect only group A streptococci, whereas acute pharyngitis is sometimes caused by group C or G streptococci, and these may require antibiotic therapy. The other is cost: A blood agar plate currently costs about $0.25, whereas EIA tests cost about $2.00 each, and optical immunoassays cost somewhat more. Although some RADTs may be waived as "simple" laboratory tests, this may not exempt the physicians laboratory from compliance or certification under the US Federal regulations adopted in the Clinical Laboratory Improvement Act (CLIA).

Particle agglutination methodologies have been employed for detecting group B streptococcal antigens in CSF or urine. Testing of urine samples has been discouraged because the urine sample may be positive as the result of skin or genitourinary contamination, or possibly from intestinal absorption and urinary excretion of antigen.[31] Many laboratories have discontinued antigen testing of CSF because it generally offers no better sensitivity than a properly performed Gram stain. No rapid tests have proved sensitive enough to determine maternal colonization directly from vaginal swabs. However, sensitive PCR test is now coming into use.[32]

3.4.1.5. Definitive Identification. Streptococci are classified into Lancefield groups on the basis of their cell wall carbohydrate antigens. The classical methods of grouping employ an extraction step, usually hot acid (Lancefield method) or nitrous acid, followed by detection of liberated antigens with specific rabbit antisera. More recently, latex and similar particle agglutination kits make it possible for any laboratory to provide rapid and accurate group identification without having to maintain serologic supplies. Molecular methods are now replacing some of the more tedious procedures.

Classic typing of group A streptococci is done by precipitation of M and T proteins with specific antisera, as described by Lancefield in 1928.[5] The M protein is the major antigenic surface marker, and antibodies to its variable N-terminal region are protective both in humans and in mouse protection tests. The T proteins are trypsin-resistant antigens that are not associated with protection but are useful markers for certain M types and for strains having no detectable M protein. T typing is done first by agglutination after trypsinization of the streptococcal cells. M typing is then done on the basis of T typing results, after an acid extraction step and precipitation with specific antisera. For example, T1 strains are invariably M1, and T3 strains are M3, whereas T8/25/Imp.19 strains may be M2, 55, or 57. About 93 M types are internationally recognized, but a quarter or more of isolates are not typeable with either T or M antisera. The major technical difficulties with this system are the difficulty in making antisera and validating new M types by functional protection testis. Testing for many of the less common but established M types cannot be done simply because typing sera are no longer available.[33] One method to overcome these problems is a surrogate typing system based on the neutralization of opacity factor (OF) described by Fraser and Maxted in 1979 and later modified by Johnson and Kaplan.[34] This test is relatively simple and correlates well with M typing. A second surrogate for M typing is "*emm* typing," which is based on the DNA sequences of putative M protein genes, described in the next section.

The epidemiologic importance of group A typing is obvious, since certain M types are commonly associated with specific kinds of infection or sequelae. Table 3 lists the most common M types associated with uncomplicated pharyngitis, severe systemic infections, and rheumatic fever, and with acute glomerulonephritis.[35–37] Molecular studies have confirmed these observations and further identified *emm*-type patterns particularly associated with skin infection.[38] M1 strains are common in both uncomplicated pharyngitis and in severe invasive disease, but epidemiologic evidence suggests that the serious disease is caused by especially virulent strains or clones, and virulence is not always associated as a general property of that serotype.[36] Molecular methods, described below, now confirm such observations and shed light on the evolution of virulent clones and the virulence genes themselves.[39–41]

The typing of group B streptococci is based on the detection of capsular polysaccharides, listed in Table 4. There are four "classical" types: Ia, Ib, II, and III; two newly recognized types of growing importance: IV, V; three new relatively uncommon types: VI, VII, VIII; and a newly proposed type IX.[42,43] In her original description of the type polysaccharides, Lancefield noted that, finding no antigens analogous to the M proteins of group A streptococci, she was looking for capsular materials like those found in the capsules of pneumococci.[5] The group B indeed proved to have capsular polysaccharides, and are all similar in structure,

Table 3. Group A Streptococcal M Protein Serotypes Commonly Associated with Uncomplicated Pharyngitis, Scarlet Fever, Severe Systemic Infections, Acute Rheumatic Fever, and Acute Glomerulonephritis, Listed in Approximate Order of Frequency[a]

Uncomplicated pharyngitis	Scarlet fever	Severe systemic infections	Acute rheumatic fever	Acute glomerulonephritis
1	3	1	3	2
12	4	3	1	1
4	1	18	18	49
2	22	12	5	12
28	6	22	4	4
3	2	5	6	55

[a]Data from Dillon et al.,[35] Johnson et al.,[36] and Colman et al.[37] Severe infections included streptococcal toxic shock, septicemia, pneumonia or empyema, joint, and deep soft tissue infections. Note that strains associated with acute glomerulonephritis may be isolated from throat (M1 and M12) or skin sites (M55) or either throat or skin (M4, M49).

Table 4. Type-Specific Antigens and Cellular Components of Group B Streptococci

Capsular polysaccharides	Serotypes Ia, Ib, II, III, IV, V, VI, VII, VII, IX (proposed) Protects organism against phagocytosis
c Protein	Occurs with all type Ib, many type Ia (designated Ia/c), about 50% of II (II/c), and some type III strains Binds to the Fc portion of IgA
β-Hemolysin	Pore-forming cytolytic toxin involved in cell invasion and injury, also confers orange pigmentation to colonies
CAMP factor	Enhances sphyngomyelinase activity (CAMP test)
Hyaluronate lyase	Degrades hyaluronan and chondroitin sulfate in basement membranes, presumably facilitating invasion
Degradative enzymes	C5a peptidase inactivates complement-mediated chemotaxis; extracellular peptidases, nucleases, collaginases, serine protease

composed of glucose, galactose, and *N*-acetylglucosamine. Unlike pneumococci, they all have side chains containing terminal sialic acid residues that are major antigenic determinants. There are a large number of surface protein antigens of pathogenic and immunological importance.[44] Type Ib strains, about half of type II and some type III strains also carry the "c protein," a complex antigen that binds the Fc portion of IgA. The R antigens, which are resistant to trypsin, occur on some type II and III strains but are not frequent among human isolates. The X antigen is found almost exclusively in veterinary strains, particularly those from bovine mastitis.

The pneumococci are classified into about 90 distinct types based on antisera to their capsular polysaccharides. The Danish typing system recognizes 48 main types or groups of closely related types. (Each of these corresponds to an individual type of the American system, which is no longer in common use.) For example, types 1–5 each contain only one distinct type. Group 6 includes type 6A (America type 6) and type 6B (American type 26) and a recently identified type 6C.[45] Knowledge of the distribution of types in human infections led directly to the selection of the most important types in the formulation of pneumococcal vaccines.

Group D and viridans streptococci are usually speciated by their pattern of biochemical reactions. Various test kits and automated equipment are in general use in most clinical laboratories and give basically similar results. However, these products do not always conform to the latest taxonomic changes. Two major identification schemes are currently in use, one from Great Britain, the other from the CDC in the United States. They differ mainly in the classification of group F and the *S. anginosis* ("*milleri*") group. This group currently consists of three species: *S. anginosis, Streptococcus constellatus,* and *Streptococcus intermedius,* which differ in their production of β-glucosidase, hyaluronidase, and several other glycosidases.[24] A group of organisms formerly described as "nutritionally deficient streptococci" have been reclassified into two new genera, *Abiotrophia* and *Granulaticella*, on the basis of 16S ribosomal RNA similarities.[24] These organisms are noteworthy as a cause endocarditis.[46] *Streptococcus mutans* is divided into eight serologic types based on cell wall carbohydrates, but epidemiologic

studies now favor molecular methods for distinguishing strains among types.[8]

Most enterococci share a glycerol teichoic acid antigen with the group D streptococci. However, enterococci are usually characterized by their ability to grow in broth containing 6.5% NaCl and hydrolyze esculin in the presence of 40% bile salts (bile-esculin medium), and a positive PYR reaction. Strains are divided into five major groups and 33 species mainly on the basis of biochemical tests. *Enterococcus faecalis* is distinguished from *Enterococcus faecium* by growth in the presence of tellurite, reduction of tetrazolium, and fermentation of sorbitol.[25,47]

3.4.1.6. Molecular Methods. Molecular methods have now been applied to various streptococcal species for direct detection, identification, epidemiology, and studies of molecular evolution. Although it is unlikely that the polymerase chain reaction (PCR) will ever completely replace the blood agar plate, hybridization with labeled gene probes has been successfully employed for detection of group A streptococci from throat swabs of patients with pharyngitis.[48] A rapid PCR test has been developed for detection of group B streptococcal colonization directly from swabs in pregnant women.[32] Pneumococcal DNA in materials such as blood cultures and middle ear fluids has also been demonstrated by PCR, using primers derived from known nucleotide sequences from genes coding for autolysin or pneumolysin.[49]

Molecular identification of group A streptococci M protein genes, called *emm* typing, has now essentially replaced classical serologic M typing.[50,51] This method is based on DNA sequencing of the N-terminal end of the gene, which codes for the highly variable antigenic portion of the M protein generally detected by specific antisera. In addition to the 93 classically validated M types, 110 officially designated *emm* types have been described, along with many more provision types and subtypes. The CDC maintains a dedicated, curated web site and searchable database of validated *emm* types, provisional types, and subtypes (http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm). This has been especially useful for epidemiological tracking of otherwise nontypeable strains. It also has the advantage that *emm* types can be identified in isolates that express only small amounts of M protein and are only weakly antigenic in conventional M typing.[33] Nevertheless, the *emm* typing is not without its own set of difficulties. Many strains have several *emm* or M protein-like genes that may look like M protein genes but code for proteins with lectin-like functions, such as binding of immunoglobulins or fibronectin, and some appear to be extra copies or even defective genes. The amplification and/or detection of these genes may depend greatly upon the selection of primers and the conditions of the assay.

Several other molecular approaches differentiate among streptococcal strains or types but are not necessarily intended to correlate with given M or T typing systems. "Ribotyping" is based on the pattern of amplified ribosomal RNA in agarose gel electrophoresis or by automated systems. Depending upon the primers utilized, species-specific and sometimes strain-specific identification may be possible. Because ribosomal RNA nucleotide sequences are highly conserved, few differences may be seen even between strains of differing M types.[52] Restriction fragment length polymorphism (RFLP) relies upon endonucleases, such as *Sma*I or *Sfi*I, that cut DNA at relatively fewer sites, producing longer more distinctively sized fragments. These large (20–500 kb) fragments must be resolved using pulse field gel electrophoresis, hence the more familiar name, PFGE. PFGE has proved useful in a wide variety of epidemiological studies.[53] Various methods similar to those just described have been employed for epidemiological studies of group B[54] and group G[55] streptococci, pneumococci,[56] and enterococci.[57,58] Molecular methods have also focused on genes of particular interest because of their function in disease, antibiotic susceptibility, or epidemiology. Two highly pathogenic clones of group A *Streptococcus* were tracked and delineated by the allelic variants of their scarlet fever toxin (SPE-A), serotyping, and multilocus enzyme electrophoresis (MLEE).[59]

Two new molecular typing methods have come to the fore over the past decade: MLST—multilocus sequence typing and MLVA—multilocus variable-number tandem repeat analysis. MLST was proposed in 1998 as a universal definitive and portable method characterizing bacteria.[60] Selected internal fragments of about 500 bp in length are amplified from seven different "housekeeping" genes, which are relatively stable genetic markers. The sequence of each locus is assigned a different number, whether it represents a single point mutation or a large recombinatorial replacement. MLST methods are in use for group A *Streptococcus*,[61] group B *Streptococcus*,[62] *S. pneumoniae*,[63] *S. suis*,[64] and *E. faecium*.[65] These techniques have been used to track clonal spread of group A streptococci resistant to multiple antibiotics,[53] to characterize nontypeable group B streptococci,[66] to study the population biology of pneumococci,[67] and to assess both nosocomial[58] and global spread[57] of vancomycin-resistant enterococci.

MLVA methods depend on PCR amplification of selected gene segments that contain a variable number of short repeated sequences. These segments vary among isolates and yield gene products of different lengths that can be separated by electrophoresis on agarose gels. The banding patterns (done individually or in a multiplex) are highly reproducible and most systems have powers of resolution equal to that of MLST. MLVA is generally cheaper, much

easier to perform, but more suitable for short-term outbreak investigations than for long-term population studies, compared to MLST. In some systems each allele associated with given locus is assigned a number corresponding to the number of tandem repeats, thus giving a numerical repeat profile that can be compared without reference to the gel pattern itself.[68] MLVA methods have been applied to pneumococci,[69] *E. faecalis*,[68] and *E. faecium*.[70] However, the current MLVA typing scheme for *E. faecium* was found to be somewhat less discriminatory than MLST and PFGE for hospital studies.[71]

3.4.2. Serologic and Immunologic Diagnostic Methods. Antibody tests have been developed as clinical and epidemiologic tools in the study of group A, group B, and pneumococcal infections. In general, antibodies are markers of past experience with the organisms and do not indicate when an infection took place. For this reason they are most useful when acute and convalescent antibody levels are compared in relation to an episode of presumed infection.

Assays for the group A *Streptococcus* are based on the development of antibodies either to cellular antigens or to extracellular enzymes. These are listed in Table 5 and further described in Section 4. Tests for antibody to M proteins, which confer immunity, and to the group A carbohydrate have been used primarily for research purposes. Assays used clinically for confirmation of recent infections have been reviewed by Shet and Kaplan.[72] The antistreptolysin O (ASO) is the most reliable and is widely available. It is of no immediate value in the diagnosis of acute streptococcal pharyngitis, and it should not be expected to differentiate carriage from infection. Nevertheless, about 80% of patients with rheumatic fever or pharyngitis-associated acute glomerulonephritis infection will mount a significant ASO response. A rise in titer is usually seen 3–6 weeks after infection, and a rise, even if modest, is more helpful than a single determination. Test kits give a titer of >166 Todd units as elevated for adults, but there is considerable variation in the "normal" values among populations and laboratories. In general, single ASO titers above 250 in adults and above 300 in school-aged children are considered elevated. Compared to pharyngitis, skin infections tend to elicit feeble ASO responses but greater responses to DNase B. The anti-DNase B titer peaks later, at 6–8 weeks after either skin or throat infections. This test is often useful when the initial ASO is low or negative. There is less clinical experience with the anti-hyaluronidase and anti-streptokinase tests, although they may give comparable results and may be useful as confirmatory tests. The Streptozyme® hemagglutination test (Wampole Laboratories, Stamford, CT) is a crude screening test based on reactions with *a mixture of streptococcal antigens, including streptolysin O, DNase, NADase, streptokinase, and hyaluronidase.* It is simple and widely available but is not considered sufficiently reliable by many authorities. Positive responses appear earlier (1–2 weeks) and should always be confirmed with one or more of the standardized assays whenever rheumatic fever or acute glomerulonephritis is suspected.[30]

Table 5. Group A Streptococcal Antigens and Antibodies[a]

	Antibody	Clinical interpretation
Cellular antigens		
M protein	(M protein)	Type specific, confers immunity. Some antigens cross-reactive with sarcolemma of heart muscle
Group A carbohydrate	(Group A CHO)	Slow response following infection
Hyaluronic acid capsule	None	Not antigenic
Extracellular enzymes		
Streptolysin O	Antistreptolysin O (ASO)	Increases after most group A infections, more reliable for throat than skin infections
Deoxyribonuclease A, B, C, D	Anti-DNase B	Most useful test for skin infections, also reliable for throat infections; anti-DNase A and C inconsistent
Hyaluronidase	Antihyaluronidase	Increases after most group A infections
Streptokinase	Antistreptokinase (ASK)	Increases after most group A infections; streptokinase A more common than B
Nicotinamide adenine dinucleotidase	Anti-NADase (anti-DPNase)	Response better after throat than skin infections; common to groups A, C, and G streptococci
Proteinase	Antiproteinase	Antibodies appear in small amounts following infection with group A
Erythrogenic toxins A, B, and C	Antierythrogenic toxin	Toxin produces rash of scarlet fever; Dick skin test for immunity or susceptibility

[a] Adapted from Quinn.[1]

Assays for antibody to pneumococcal and group B streptococcal capsular polysaccharides have employed radioimmunoassay (RIA) for total antibody and enzyme-linked immunosorbent assays (ELISA) for total and class-specific antibody determinations. Since both pneumococci and group B streptococci have multiple capsular types, antibody to specific types must be considered. Although it has so far proved impossible to establish an absolute or minimal protective antibody level, infection appears to be more common in subjects with low antibody levels against the specific capsular antigen of the type causing infection. Assays for type-specific pneumococcal antibodies are now commercially available.

4. Biological Characteristics of the Organisms

The various streptococcal species have many biologic similarities and differences. The genus name suggests a "twisted chain," which describes the microscopic appearance of many species, especially when grown in broth culture. The pneumococci are commonly described (and were formerly named) as diplococci because of their propensity to occur in pairs, but they are often indistinguishable from other streptococci in blood cultures. All the streptococci have a tough cell wall composed of cross-linked peptidoglycans. Most have a polysaccharide group antigen associated with the cell wall, and some have teichoic acids as major or additional components. Pneumococci exhibit prototypic bacterial polysaccharide capsules. The group A streptococci, in contrast, have the M proteins on their exterior surface, but these appear to play a similar role in helping the organisms resist phagocytosis. The group B streptococci, like pneumococci, have polysaccharide capsules as their major surface antigens. Pneumococci, however, also have autolytic enzymes that break down cell walls in late growth phases, releasing DNA and other intracellular components. Intact pneumococci also take up genetic material and are thus auto-transformable, a characteristic that appears to have facilitated the spread of antibiotic resistance within the species. With few exceptions, streptococci are aerobic and facultatively anaerobic. They are cytochrome-negative, catalase-negative, and ferment sugars mainly to lactic acid but not to gas. All streptococci secrete enzymes extracellularly, but those of group A have been studied most extensively.

4.1. Cellular Antigens and Enzymes

4.1.1. Group A Streptococcal Cellular Antigens and Enzymes. The major components of group A streptococci are the cellular antigens and the extracellular enzymes listed in Table 5. M proteins, noted above and listed in Table 3, are important virulence factors of group A streptococci, contributing to the organism's resistance to phagocytosis in the absence of type-specific antibody.[73] M proteins bind host proteins, especially fibrinogen, as a ploy to evade host defense mechanisms. Although immunity appears to be lifelong, most humans are usually infected by only a few different types and remain susceptible to the other types. Thus, repeated episodes of streptococcal infection may be due to different types rather than to a failure of host response. Certain M types, especially M1 and M3, have been associated with more severe forms of disease, and some are more common in pyoderma and acute glomerulonephritis[4,36] (see Table 3). Others have historically been associated with acute rheumatic fever and are referred to as "rheumatogenic" types. Although rheumatogenicity is not determined by M type alone, evidence for the cross-reactivity of certain M proteins with heart and brain tissue strongly suggests autoimmune mechanisms for the etiology of acute rheumatic fever and Sydenham's chorea.[74,75]

The group A cell wall carbohydrate is a polymer of rhamnose with *N*-acetylglucosamine side chains. Humans normally make antibodies to this antigen but the role of antibodies has been controversial. There is now evidence that this antigen may play a role in sequelae of streptococcal infections by inducing antibodies cross-reactive with cytokeratin.[75] This could provide an explanation for the joint and skin manifestation of acute rheumatic fever and of guttate psoriasis. Vaccinating mice with group A carbohydrate cross-linked to tetanus toxoid appears to induce antibodies that are protective against challenge with group A streptococci.[76]

Hyaluronic acid capsules are produced by some (especially M18) strains, giving colonies a large highly mucoid appearance. This capsule material is indistinguishable from the ground substance of mammalian connective tissue and is not immunogenic. Its effect on virulence in mice is small, although similar capsules may occur on group C streptococci and have greater virulence than unencapsulated strains. Nevertheless, mucoid group A strains have been associated with severe disease in humans and with rheumatic fever.[36,74] Furthermore, the near-disappearance of acute rheumatic fever in the United States may be correlated with the replacement of rheumatogenic M types by nonrheumatogenic types in cases of pharyngitis.[77]

Lipoteichoic acids are composed of polyglycerophosphate attached to lipids. These surface molecules are directly involved in attachment of organisms to host epithelia and are of importance in the initiation of infection.[78] Other cellular components are less well defined in terms of their role in disease. As in other gram-positive bacteria, there is a rigid

cell wall structure made from polymers of alternating glucosamine and muramic acid units cross-linked by peptide side chains. This serves to stabilize the organisms against outside osmotic changes. The peptidoglycan components are highly inflammatory and may play a role in inciting nonspecific host responses. The T proteins, noted above, occur in families that may be shared by a number of M types. The serum opacity factor proteins are coexpressed with specific M types and are not shared among M types.[34] The R antigen is an antigenic surface protein that occurs in strains of various types but appears to play no role in virulence or protection. Like groups C and G streptococci, group A and other streptococci also have antibody-binding proteins that bind antibodies nonspecifically via the Fc fragment, presumably to help the organism avoid specific, complement-fixing, antibody binding.[79]

Group A streptococci secrete various substances into the surrounding milieu that may contribute to the pathogenic process. There are two well-described hemolysins capable of lysing red blood cells and injuring other cell membranes and subcellular organelles.[80] Streptolysin O is the antigenic, oxygen-labile hemolysin used in the ASO test. Streptolysin O is a pore-forming toxin that facilitates the entry of NADase and presumably other toxins into cells.[81] Streptolysin S is nonantigenic, oxygen stable, and is responsible for β-hemolysis at the surface of cultures grown on blood agar under aerobic conditions. This hemolysin acts as an epithelial cell toxin and also impairs phagocytic clearance of the organisms.[82]

Deoxyribonucleases (DNases) are elaborated by groups A, B, C, and G streptococci. DNase B is the most common and most immunogenic of the group A DNases and is the basis of the antibody test of the same name. It is thought that these enzymes along with hyaluronidase and streptokinase combine to produce the thin pus seen in streptococcal infections, in contrast to the thick pus often associated with infections due to other pyogenic bacteria.[1] The pathogenic role of DNases now appears to be aid in evasion of innate host defense mechanisms.[83]

Streptococcal hyaluronidases (produced by groups A and C) are capable of hydrolyzing the hyaluronic acid of group A capsules and of mammalian connective tissue. Although formerly called "spreading factor," its biologic role remains uncertain with regard either to cell metabolism or to the production of disease. It appears that hyaluronidase does facilitate spread of large molecules but not bacteria in the area of infection, but its major role may be to enable the organism to utilize hyaluronic acid as a carbon source.[84]

The streptokinases are antigenic proteins that convert plasminogen to plasmin, which in turn lyses fibrin clots. Group A streptococci produce either streptokinase A, the most common, or streptokinase B. An antibody test based on the former antigen is sometimes employed in the clinical assessment of group A disease. A distinctive low-molecular-weight streptokinase, called "nephritis strain-associated protein," has also been identified from group A streptococci recovered from patients with acute nephritis.[85]

Nicotinamide adenine dinucleotidase (NADase; also called diphosphopyridine nucleotidase, DPNase) is produced by streptococci of groups A, C, and G. Anti-NADase antibodies are produced by the majority of patients recovering from group A streptococcal pharyngitis, but responses are poor following skin infections. NADase is toxic to leukocytes but requires pores formed by streptolysin O in order to enter cells and enhances the virulence of the organisms in vivo.[81] Recent studies have found NADase production in all strains of group A streptococci isolated from invasive cases and have demonstrated that the current expression of NADase in M-1 strains correlated with the temporal emergence of M-1 strains associated with invasive disease around 1985.[86]

Several proteinases of group A streptococci have been carefully studied and shown to exert pathologic effects in vitro and in vivo. A specialized peptidase has been described that diminishes chemotactic activity by inactivation of the C5a complement component. *Streptococcus pyogenes* exotoxin B (SpeB) is a cysteine protease that cleaves the hinge region of human IgG and has other immunomodulating properties.[87]

Streptococcal pyrogenic exotoxins (SPEs) are the erythrogenic toxins responsible for the characteristic rash of scarlet fever. These enzymes have been implicated as factors in streptococcal toxic shock, where they appear to be potent activators of tumor necrosis factor (TNF) and other cytokines.[4,88] There are three classic antigenically distinct toxins, designated SPE A, B, and C. All group A streptococci carry a gene *(speB)* that codes for SPE B, but it is not understood why some strains are stronger producers of the toxin than others. SPE A and SPE C are encoded by lysogenic bacteriophages, and only those strains infected by the phages are capable of producing toxin. Humans make antibodies to SPE A, B, and C, which appear to confer toxin-specific immunity to scarlet fever. It is possible to have scarlet fever more than once, due to different toxins. A large family of SPEs (so far SPE D–M) have now been identified with various pyrogenic, mitogenic, and superantigen properties.[89] The classic determination of susceptibility to scarlet fever is the Dick test.[1] Seldom used today, it is based on the observation that patients with antibody to a specific toxin show no response to a small intradermal injection of that toxin (negative Dick test). Susceptible individuals, who have no

antibody to neutralize the toxin, develop inflammation at the injection site within 24 h (positive Dick test). SPE A shares structural and physiologic similarities with TSST-1, one of the toxins associated with staphylococcal toxic shock syndrome (see also Section 7.1).

4.1.2. Group B Streptococcal Cellular Antigens and Enzymes. The group B streptococci differ from group A in that their virulence may be accounted principally by capsular polysaccharides rather than proteins. The capsular types, noted in Section 3.4.1 and in Table 4, are antigenically distinct by virtue of variations in linkages of the same essential sugars. A key feature is that all have terminal *N*-acetylneuraminic acid (sialic acid) residues that are major immunodeterminants. The capsules are antiphagocytic and require specific antibody for efficient opsonization. The quantity of sialic acid-containing antigen appears to be directly related to size and density of the capsule and to virulence in animal models.[90,91] The capsular material itself appears to inhibit the activation and chemotactic functions of neutrophils.[92] Many group B strains are also capable of binding fibrinogen to their surface in a manner that competes with the nonspecific binding of C3 complement.[87,92]

The group B antigen is a complex glucitol-containing polysaccharide associated with the peptidoglycan cell wall. Antibodies to the group B antigen are generally not protective, presumably because it is covered by capsular material. A human monoclonal IgM antibody to the group B antigen has been described that opsonizes strains of all serotypes,[93] but the large amount of antibody required appears to make impractical as an adjunctive therapeutic agent.

The major surface structures and protein antigen of group B streptococci have been reviewed by Lindahl and colleagues.[44] The major protein to be studied was the c protein, which occurs on all type Ib and some type II and III strains. Antibodies to this antigen are protective,[94] but common variants of the protein apparently confer resistance to intracellular killing by neutrophils. An important property of the beta c protein may be its ability to nonspecifically bind human IgA.[92] The alpha c protein is involved with adherence to epithelial cells via a glycosaminoglycan-binding region and mediates entry in host cells.[95] The R and X antigens are rarely seen in human isolates and probably play no role in protection or disease. Also present are lipoteichoic acids (LTA) that resemble the lipopolysaccharides (LPS) of gram-negative bacteria and engage the Toll-like receptors (TLR-2) of the innate defense system.[96]

The group B streptococci elaborate a number of extracellular enzymes, including hemolysins, CAMP factor, DNases, and "neuraminidase." Pritchard et al. have shown that the enzyme thought for many years to be a "neuraminidase" is in fact a hyaluronic acid lyase that has a unique mechanism of action quite unlike that of hyaluronidases produced by group A streptococci or pneumococci.[97] Group B streptococci have several hemolysins, one of which has been identified as cytotoxic for mammalian cells in vitro and inhibited by phospholipids common to pulmonary surfactants.[80] The CAMP factor potentiates the activity of staphylococcal sphingomyelinase in vitro but is not essential for systemic virulence.[98] Like group A, group B streptococci elaborate a proteinase that decreases chemotactic activity by specifically cleaving complement C5a.[44,99] A pyrogenic exotoxin has also been identified from strains associated with a group B streptococcal toxic shock-like syndrome in infants.[100]

4.1.3. Pneumococcal Cellular Antigens and Enzymes. *Streptococcus pneumoniae* is the paradigm of encapsulated bacteria. Its polysaccharide capsules are essential to virulence and antibodies against the capsule are the major specific defense against infection. The 90 recognized type-specific polysaccharides vary in composition, including linear polymers, branched chains, and teichoic acid-like antigens. The most frequently occurring types have been selected for inclusion in the presently licensed vaccines.[101]

The C-polysaccharide corresponds to the group carbohydrates of other streptococci but differs significantly in structure. Its major antigenic determinant is phosphocholine, linked to ribitol phosphate, galactosamine, and other sugars. Although humans make "natural" antibodies to this antigen, opsonization of pneumococci (and presumably protection) is almost entirely dependent upon anticapsular antibodies.[102] The Forssman antigen is a membrane teichoic acid similar to C-polysaccharide but linked to a lipid, forming what is essentially the lipoteichoic acid of the pneumococcus.

Pneumococcal surface protein A (PspA) has been identified on essentially all important clinical isolates and appears to play an important role in virulence. Humans including young infants make antibodies to PspA and to various other surface proteins, suggesting their use in a vaccine.[103] Enzymes produced by pneumococci include pneumolysin, amidase (the autolytic enzyme that breaks down cell wall material), neuraminidases, and IgA proteases. Pneumolysin is a pore-forming toxin that bears extensive amino acid sequence homology to streptolysin O and to the theta-toxin of *Clostridium perfringens.* It is highly toxic to pulmonary epithelial cells and may be important to the pathogenesis of pneumonia.[101]

4.1.4. Components of Other Streptococci. Other pyogenic streptococci share many characteristics noted above. All have rigid peptidoglycan cell walls, with various

distinctive or group antigens, and usually with some form of lipoteichoic acid. Group C streptococci may have hyaluronic acid capsules like those of group A. Group G streptococci may have the type 12 M protein of group A or similar surface proteins, as well as antibody-binding proteins. The group C streptococci from human, equine, and porcine sources produce species-specific streptokinases that are otherwise similar to those of group A.[104] A streptokinase derived from group C has been used clinically in attempts to clear clotted intravascular catheters, to lyse pleural adhesions in patients with lung infections, and to help remove clots in patients with coronary artery occlusions. Group G streptococci also produce streptokinases. We have described a patient with nephritis following infection with a group G strain that had a low-molecular-weight enzyme similar to the nephritis strain-associated protein of group A.[105]

The enteric and oral streptococci are usually unencapsulated. Some in the *S. anginosis* (*milleri*) group appear to have capsules more often when found in abscesses, and the capsules play a role in protection from phagocytosis.[106] Few toxins or noxious enzymes have been described among the enteric and oral streptococci, but this may be from lack of concerted investigation. Members of the *S. anginosis* group have, at the least, hyaluronidase, deoxyribonuclease, and various proteinases.[24,106] For many of the less virulent streptococci, the inflammatory response to infection probably relates more to the properties of the cell wall breakdown products than to specific enzymes or toxins. Characteristics that enable them to cause disease often relate to their ability to adhere to host tissues, such as tooth enamel, heart valves or prostheses, or to intravascular catheters. *Streptococcus mutans,* for example, adheres to the pellicle coating the tooth surface by specific protein receptors called antigen I/II; adhesion is further facilitated by the presence of sucrose. *Streptococcus mutans* also produce extracellular proteases that are capable of breaking down cemental collagens and other host substrates. Caries occur when the secretion of acids demineralizes the enamel and organisms adhere to and invade the tooth surface. *Streptococcus mutans* also produce mutacins, bacteriocin-like antibiotic peptides, that help protect their ecological niche in dental biofilm.[9,107] Enterococci and *S. anginosis* group streptococci are frequently found in mixed infections, especially in association with anaerobic bacteria, suggesting that additional factors are required for them to cause disease. Enterococci produce several pheromones that are chemotactic for neutrophils and may contribute to the inflammation associated with infection. *Enterococcus faecalis* also produces a plasmid-encoded hemolysin. Because enterococci are frequently resistant to common antibiotics, serious enterococcal disease also occurs as superinfection in patients receiving broad-spectrum antibiotics that may disturb the normal ecology of this usually benign organism.[12]

4.2. Antibiotic Susceptibility

The streptococci are generally quite susceptible to penicillin, including most oral and *S. anginosis* group streptococci. Exceptions include some pneumococci and group D streptococci, and the enterococci. Although streptococci are generally resistant to aminoglycosides, gentamicin is sometimes used for its synergistic effect in combination with a penicillin, particularly in patients with endocarditis due to viridans streptococci and enterococci.[108] Chloramphenicol has occasionally been used in penicillin-allergic patients, but other drugs, including erythromycin, clarithromycin, and clindamycin, are considered to be superior for most streptococcal species.

Groups A and B streptococci have never developed resistance to penicillins, probably because they are not naturally transformable, as are pneumococci and enterococci. They are somewhat less sensitive to vancomycin and cephalosporins, moderately resistant to chloramphenicol, and fairly resistant to aminoglycosides, sulfonamides, and tetracycline. Group B strains have shown some tolerance to penicillin, but the clinical significance of such observations is unknown. Tolerance to penicillin has also been suggested as one mechanism by which group A streptococci persist after treatment of pharyngitis. But although there may be a modest increase in efficacy of cephalosporins compared to penicillin, the explanation appears to be the more efficient eradication of the carrier state in patients whose pharyngitis was actually due to viral causes.[109,110] Another mechanism is thought to be the protection of susceptible streptococci by the production of β-lactamases by other bacteria in the pharynx or tonsils,[111] although this concept has been disputed.[112] Another explanation is that the streptococci penetrate the epithelial cells where they may persist even in the presence of extracellular penicillin.[113] Resistance to erythromycin occurs in about 5% of group A strains in the United States and most other parts of the world. However, local rates from 25 to 50% have been reported in Korea, Italy, and Pittsburgh, Pennsylvania. Resistance rates parallel antibiotic use and may be reduced by improving prescribing practices.[114,115]

Penicillin resistance in pneumococci has emerged slowly over the past two decades and has now become a frequent and serious problem worldwide. Most of these strains have intermediate susceptibility (MIC 0.1–1.0 μg/ml) but highly resistant strains (MIC >1.0 μg/ml), initially reported from South Africa and Spain, have now spread throughout Europe and North America. The widespread use of fluoroquinolones,

macrolides, and azithromycin has led to increased resistance to those drugs in many areas.[116] Of considerable concern is the recent emergence of strains with very high resistance to penicillin and of multiresistant serotype 19A strains, because they are not included in the 7-valent conjugate vaccine (which contains type 19F).[117]

Although group B streptococci remain sensitive to penicillin, some strains can be penicillin tolerant, which has been speculated as a cause of treatment failure in cases of invasive disease.[118] Another problem is the increasing resistance to erythromycin and clindamycin, which are used as alternatives to penicillin in antibiotic prophylaxis for allergic women.[27] Resistance mechanisms to macrolides and clindamycin have been identified as methylase genes erm(B) and erm(TR) and efflux genes mef(E) and mef(A).[119]

Enterococci are moderately resistant to penicillins alone, because of the intrinsic properties of their penicillin-binding proteins. *Enterococcus* strains with resistance to β-lactam antibiotics and high-level resistance to aminoglycosides began to appear in the 1980s. Some strains of *E. faecalis* have also acquired β-lactamases as a mechanism of resistance.[120] Infections are usually treated with penicillin or ampicillin plus an aminoglycoside, which exert a synergistic effect against the organisms. Vancomycin-resistant enterococci (VRE), especially *E. faecalis,* made their appearance in the late 1980s and have been an increasing problem ever since.[57,121] Vancomycin acts by binding to the D-alanine–D-alanine on the end of cell wall muramic acid precursors. Resistance conferred by a plasmid or transposon carrying the *vanA* or similar gene, allows the organism to generate muramic acid precursors with peptides ending in D-alanine–D-lactate, to which the vancomycin has low affinity. VRE present difficult therapeutic challenges, because there are few alternatives to vancomycin.[12,57] Most VRE are *E. faecium*, which is sensitive to quinupristin–dalfopristin, but *E. faecalis* is resistant. Linezolid is effective against both species, but as more linezolid is being used, resistance has started to appear.[58]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Group A streptococcal pharyngitis is one of the most common acute bacterial infections. The frequency of this disease, especially as manifested by scarlet fever, has declined dramatically since the beginning of the century, as illustrated in Figure 1.[1] The severity of the disease, reflected by mortality rates, declined concomitantly. This trend began long before the advent of antibiotics, suggesting a decrease in virulence or an increase in host resistance, or both. In the 1940s penicillin became widely available, and deaths attributed to scarlet fever and puerperal sepsis, the two most common lethal forms of group A streptococcal disease, became a rarity. The number of reported cases of streptococcal sore throat increased during the 1950s and 1960s, probably because of increased physician awareness of its relation to rheumatic fever, greater use of throat cultures, and the availability of antibiotics for treatment and prevention. Since the 1960s only one population-based study of streptococcal sore throat in an industrialized country has been published, a prospective study of 202 Australian families with at least one child in the 3- to 12-year age group.[122] The incidence of culture-positive cases was 13 per 100 child-years and was eight per 100 child-years for serologically confirmed group A streptococcal infection. This was similar to previous studies in suburban populations, but rates were about half that seen in crowded lower socioeconomic settings and lower still that in present day indigenous populations.[122]

Figure 2 shows the number of positive throat cultures and number of cases of acute rheumatic fever and acute glomerulonephritis in private pediatric practices participating in surveillance studies in Rochester, New York, over a 20-year period. These data were compiled and kindly supplied by Caroline Breese Hall, University of Rochester. Of approximately 23,000 throat cultures done annually, 18–25% were positive for group A streptococci in this relatively stable population. Meanwhile, the number of cases of acute glomerulonephritis declined from nearly 40 in 1967 to an average of one case per year from 1981 to 1988. Confirmed cases of acute rheumatic fever dropped from 20 to 28 per year to a very few from 1975 to 1985 and have continued to be very low. For example in Baltimore, between 1960 and 1964, the incidence of rheumatic fever was 26 per 100,000 among 5- to 19-year olds. By 1980 the rates had fallen to 0.2–0.8 per 100,000 nationwide among whites, but with rates several times higher among other ethnic groups. Beginning in the mid-1980s, an increase in new rheumatic fever cases was seen in Utah, Pennsylvania, Ohio, New York, and other areas. Disease incidence peaked in Utah in 1985 with 18 per 100,000 population age adjusted for 5- to 17-year olds,[3] and rates peaked again in 1998 and have subsequently declined.[123] Of particular interest was the observation that throughout this period incidence rates were essentially unchanged in Hawaii and New Zealand, where the disease is especially prevalent among Polynesian children. The explanation may be the local prevalence of certain rheumatogenic M protein types many of which were characteristically mucoid, whereas in other areas of the United States these have been largely replaced by nonrheumatogenic types.[77,123]

There has not been a similar decline in rheumatic heart disease in the developing world.[23] In a program begun in 1984 the WHO Cardiovascular Disease Unit surveyed 16 developing countries and found rates averaging 220 cases per 100,000 childhood population. Highest rates were in Africa and the eastern Mediterranean, lowest rates were in Southeast Asia and the Western Pacific. More recent data have found that prevalence rates of clinically diagnosed rheumatic heart disease have not changed in Cambodia in 2001–2002 (220/100,000) and Mozambique in 2005 (230/100,000). However, echocardiographic screening for mitral or aortic regurgitation revealed that 10 times as many children were affected.[124] The major problem in the developing world is establishing and maintaining effective primary and secondary prevention programs.[125] This principle applies as well to developed areas, such as Miami, Florida, where underprivileged inner-city children have attack rates of 15/100,000, compared to 0.7/100,000 for suburban middle-class children.[126]

Deaths from acute rheumatic fever are uncommon today. In developed countries deaths associated with chronic rheumatic heart disease continue to occur in persons who had acute rheumatic fever in childhood and develop severe mitral stenosis in the fourth decade or later. In 1975, for example, 9255 of 12,775 deaths attributed to rheumatic heart disease were in patients over 50 years of age.[1] The pattern is quite different in developing areas, as in South Africa, where a third of patients have mitral regurgitation usually associated with ongoing rheumatic activity.[127] Left untreated these lesions develop into a severe form of pure mitral regurgitation that requires surgery in the first or second decade of life. Degenerative valvular disease, including mitral stenosis and mixed lesions, is like those seen elsewhere but tends to occur at a younger age.

Another change in group A streptococcal disease in recent years has been the reappearance of serious acute infections, especially bacteremia and streptococcal toxic shock.[4] Since 1980, the number of invasive disease and deaths has increased in several countries. However, the rates in various population-based surveys differ depending upon whether disease was defined as toxic shock, necrotizing fasciitis, or all invasive disease. Surveillance in Ontario, Canada, revealed that the incidence of necrotizing fasciitis (NF) increased from 0.08 cases per 100,000 population in 1992 to 0.49 cases per 100,000 population in 1995, with a case-fatality rate of 13%.[128] In the Netherlands, 1994–2003, annual incidence of all invasive disease peaked in 1996 (4.0 cases/100,000/year) and was at its lowest in 1999 (2.0 cases/100,000/year).[20] Similar rates were noted from Israel.[129] In the United States 5,400 cases of invasive GAS infection (3.5 cases per 100,000 persons) were identified in 2000–2004, with 735 deaths (case-fatality rate, 13.7%). Case-fatality rates for streptococcal toxic shock syndrome were 36 and 24% for necrotizing fasciitis. Rates were highest among elderly persons (9.4 cases per 100,000), infants (5.3 cases per 100,000), and black persons (4.7 cases per 100,000) and were stable over time.[13] Chickenpox has been a major risk factor for invasive disease in children, but cases have declined since the introduction of the varicella vaccine in 1995.[130] Severe invasive infections due to group A, B, C, and G streptococci were reviewed in Denmark from 1999 to 2002. Group A infections occurred at a rate of 2–3/100,000 persons/year. Of particular note was that group G infections rose, mainly among the elderly, from 1 to 2/100,000 during the observation period. Septic shock and toxic shock syndromes were seen in group B, C, and G infections, although not as frequently as with group A streptococci.[19]

Acute poststreptococcal glomerulonephritis is currently a rare disease, as shown in data from Rochester, New York, in Figure 2. There are no contemporary data on rates for nephritis associated with either throat or skin infections in the United States, and no recent reviews. Prospective studies in Alabama (1966–1969) revealed 91 cases of nephritis relative to 1,149 cases of uncomplicated streptococcal pyoderma treated in a clinical setting, for an attack rate of about 8%.[35] Of note, however, is that outbreaks of glomerulonephritis associated with group C streptococci continue to occur, usually in association with unpasteurized dairy products.[131,132]

Group B streptococci emerged in the 1970s as the leading cause of neonatal infection. In the 1980s attack rates of early-onset disease were about 2 per 1,000 live births in defined populations in Chicago, Illinois, and Birmingham, Alabama.[26,133] Several clinical trials demonstrated the potential for prevention of early-onset disease by selective intrapartum antibiotic prophylaxis, which led to the first guidelines issued in 1996 by the CDC, the American College of Obstetricians and Gynecologists (ACOG), and the American Academy of Pediatrics (AAP). Two selection strategies were used: One (ACOG) relied of identification of clinical risk factors, such as prematurity, maternal fever, and prolonged rupture of amniotic membranes. The other (AAP) was based on screening women for vaginal/rectal colonization by group B streptococcal at 35–37 weeks of gestation. Those with colonization and/or risk factors were offered penicillin given intravenously during labor. This resulted in a 65% decrease in early-onset cases to about 0.5 per 1,000 live births from 1993 to 1998, where they remained unchanged through 2001. Meanwhile, further studies in the Active Bacterial Core Surveillance program found that routine antenatal screening prevented significantly more infections than the risk-based strategy.[134] Revised guidelines and updated prophylaxis regimens were published in 2002.[28] Since that

time, rates of early-onset disease have declined and additional 31% to 0.34 per 1,000 live births.[14] Rates among African-Americans have seen the largest decline but are still about twice that in the White population. Early-onset disease rates in Europe vary from 0.3 to 2/1,000 live births, and several countries have prevention guidelines.[16] Clinical studies in France and Italy have found similar reductions in attack rates using a combined culture and risk strategy.[135] Experience using a risk-only prevention in the Netherlands has resulted in only modest reductions from 0.54 to 0.36 cases/1,000 live births, and changes in guidelines have been recommended.[136] Early-onset disease rates are about 2/1,000 live births in South Africa and 0.9/1,000 in Malawi, but prevention strategies are difficult to apply in resource-limited settings.[137]

Late-onset disease, generally defined as occurring in infants older than 7 days, has remained unchanged for over a decade, with rates in the United States about 0.4/1,000 live births.[14] Prematurity is the major risk factor in late-onset disease, occurring in 50% or more of cases.[14,138] Rates are about the same in northern Italy (0.5/1,000 live births),[139] but higher in South Africa and Malawi (about 1/1,000 live births),[137] and considerably lower in the Netherlands (0.14/1,000 live births).[136]

Few data are available on maternal infections due to group B streptococci, such as bacteremia, chorioamnionitis, endometritis, and urinary tract infection. Maternal sepsis occurred in 0.5–2/1,000 deliveries in Alabama, with lowest rates associated with aggressive antibiotic use in mothers undergoing Cesarean section delivery.[140] Rates of chorioamnionitis and endometritis have declined since the introduction of intrapartum prophylaxis.[141]

Group B streptococcal infections in nonpregnant adults have increased between twofold and fourfold over the past two decades.[142] While still occurring at rates of a few per 100,000 population among young and middle-aged adults, those over 65 years are especially vulnerable, with rates of 25/100,000. Predisposing factors, such as diabetes mellitus, malignancy, and residence in nursing homes, are common. Infections of skin and soft tissue, urinary tract, and pneumonia predominate.[143]

Enterococci have been most notable in hospital settings, where endemic infection and outbreaks are common.[121] Vancomycin-resistant enterococci (VRE) now account for about 28% of nosocomial infections.[21] In a study of 24,000 blood culture recorded in 49 US hospitals, 1995–2002, enterococci were found in 9% of nosocomial blood stream infections (about 5/10,000 hospital admissions). The majority of blood isolates were *E. faecium*, of which 60% were VRE.[144] In the only population-based study of invasive VRE, in metropolitan Atlanta, Georgia, disease incidence increased from 0.9/100,000 persons/year in 1997 to 1.7/100,000 persons/year in 2000.[145] *Enterococcus faecium* accounted for over 80% of isolates, followed by *E. faecalis*, and a few *Enterococcus gallinarum* and *Enterococcus durans*. The crude 30-day in-hospital mortality was 40% and was best predicted by three factors: the Charlson comorbidity index, immunocompromise from chemotherapy, and prior antibiotic use. African American patients had a higher mortality rate, attributed largely to higher rates of chronic conditions, especially end-stage renal disease.

In terms of sheer numbers of persons, dental caries caused principally by the mutans streptococci, *S. mutans, Streptococcus sobrinus* and their relatives, probably infects more people than all other streptococci combined. However, the last 40 years of the 20th century saw a remarkable decline in dental caries worldwide, beginning in western Europe and North America.[146] Rates of DMFT (decayed, missing, or filled teeth) were commonly 10/100 surfaces at risk in the 1960s have come down to around 1/100 surfaces by 1988 and have remained relatively stable. The major decline in caries has been attributed mainly to fluoridation programs, fluoride-containing dentifrices, and school dental services. By the mid-1980s about half of school-aged children in the United States and Canadian children had permanent teeth completely free of cavities and restorations.[147] Some of these good news and further reductions in DMFT rates to 1/100 surfaces and below can be ascribed to the introduction of tooth sealants, general improvements in dental care, and changes in parental beliefs and attitudes.[148,149]

Survey methodology for measurement of dental caries is well established and periodically revised.[150,151] Since the populations of developing countries are skewed toward the younger age groups, who have fewer caries relative to the older populations in the developed world, a population-weighted mean DFMT at age 12 is used for global comparisons. By this measure WHO can monitor trends from more than 1,000 surveys submitted to the WHO Global Oral Data Bank, set up in 1970. Some developing countries have seen a rise in DFMT thought to be associated with changes in diet and other factors associated with urbanization.[150] In the absence of fluoridation and adequate dental care, increased sugar consumption may also play a role.[152] Most caries and DMFT data from the developing world now come from regional or national survey or from compilations of published reports.[153]

With the decline in caries incidence in many countries DFMT data may be somewhat less useful, especially for clinical intervention trials, in which individual counts of new decayed, missing, and filled surfaces (DMFS) over a defined period may be better indicators. The decline in caries has also had an impact on the number of patients required

for statistical power in clinical studies.[154] Newer models focus on the evaluation of caries risk, such as Cariogram, a software program that evaluates an individual's caries risk profile and illustrates it in graphic form. The predictive variable which makes the greatest contribution to the model is *S. mutans* count, followed by the DMFT index and the buffer capacity of the saliva.[155]

5.2. Epidemiology and Contagiousness

Of all the streptococcal infections only scarlet fever has been feared as an epidemic disease in the same sense as cholera and plague. In most areas of the world, group A streptococcal disease is endemic, with fluctuations exceeding the normal prevalence levels occurring seasonally or sometimes over an extended period. Localized epidemics of streptococcal pharyngitis or skin infections and acute rheumatic fever are occasionally reported from newborn nurseries, hospitals, nursing homes, day care facilities, and military installations.[156,157] An unusual nursery epidemic occurred when infants were infected from clothing that was improperly cleaned in a hospital laundry.[158] Environmental contamination is thought to be a source of spread within day care facilities, with several studies showing streptococci recovered from environmental surfaces, fabrics, and toys shared by many of the children. A large outbreak of perianal infection prompted a careful investigation that revealed a particular group A streptococcal clone associated with an attack rate of 2–7 per 1,000 children per year in a small rural community in Denmark.[159] Although several urban myths have circulated about contracting streptococcal sore throat from the family dog, household pets are an unlikely source of group A streptococcal infection.[160]

Streptococci associated primarily with the respiratory tract, such as group A and the pneumococcus, cause infections that are initially related to that portal of entry. Of course, some group A strains prefer to colonize the skin and cause impetigo, whereas others affect either skin or throat. The group B streptococci, in contrast, are gut organisms. Asymptomatic gastrointestinal, and to a lesser extent genitourinary, colonization is common. Their infections relate to the aberrations induced by pregnancy, labor, and delivery, and to the unique conditions of infants in the newborn period. The enterococci are also normal gut organisms, but their infections are more generally opportunistic in nature.

Group A streptococci are not considered part of the normal flora of the respiratory tract, and their presence is generally associated with overt infection. Transmission to other persons is greatest from an infected individual, and communicability appears to be dose related. Nevertheless, a large reservoir of asymptomatic carriers, mainly children, exists with endemic rather than epidemic characteristics.[122] Individuals identified as carriers harbor relatively small numbers of organisms. They are at little risk for developing acute disease or sequelae themselves, and they are an uncommon source of new infection.[161] However, the identification of the carrier state, usually defined by the absence of a serologic response, is fraught with difficulties that continue to cloud the relationship between colonization and disease.

Group B streptococci cause serious disease in the perinatal period, but only a small number of infections occur among colonized mothers and infants exposed in utero, during, or after delivery. Maternal group B infections may be initially subclinical and possibly contribute to premature labor or septic abortion.[162] Amnionitis may be present before the onset of fever or other symptoms. After delivery, a previously asymptomatic mother may develop endometritis, usually without bacteremia. Classical puerperal sepsis due to group A streptococci, in contrast, was often transmitted via obstetric personnel and occurred as a fulminant infection after delivery, sparing the infant. With group B streptococci, infected infants are usually exposed in utero to the serotype carried by the mother. Infants with this "early-onset" form of infection are usually bacteremic at delivery and become symptomatic within a few hours of birth.[26,163] Infection is directly related to the size of the inoculum to which the infant is exposed, by swallowing or aspirating infected amniotic fluid. Most infants are lightly colonized and at little risk for infection, whereas those who are infected are almost invariably heavily colonized at birth. Some infants may acquire organisms via the respiratory tract during transit through the birth canal, or from persons other than the mother, and become symptomatic at a later time. Development of the late-onset form of disease may be delayed for days or weeks, and only about half of these infants will be infected by an organism acquired at birth or from the mother.[26,138]

Pneumococci are often thought of as normal respiratory flora, but pneumococcal infections are not opportunistic in the sense that they are caused by any pneumococcus that happens to reside in the nasopharynx. Most infections are due to new types not previously carried by a given individual. This was first observed in the classical investigations of Hodges and MacLeod on pneumonia at a United States Air Force training facility in the 1940s.[164] Within about 6 weeks of arrival, new recruits often acquired one of a small number of "epidemic" types (1, 2, 4, 5, 7, and 12) prevalent at the base. Although the rate of carriage of the epidemic types was relatively low, the "infectivity factor" (measured by the ratio of cases of pneumonia to the number of carriers) was much higher than that of other types commonly carried. Similarly, infants in our prospective study frequently carried one of several common types for prolonged periods, but when

they develop otitis media it was usually within 4 weeks of acquiring a type they had not carried previously.[165] This epidemiologic relationship has been nicely confirmed in the Finnish Otitis Media Cohort Study.[166]

The concept of dental caries as a transmissible infectious disease was drawn from animal studies in the 1960s. *Streptococcus mutans* has been implicated as a major pathogen in caries, and it appears that most infants acquire *S. mutans* strains from their mother.[7,107] These observations have been confirmed using molecular methods in a prospective studies of acquisition and infection.[8,107] They further developed evidence for a discrete "window of infectivity" between 18 and 30 months of age, coinciding with the emergence of the 20 primary teeth. Children who did not acquire *S. mutans* during this period were at much lower risk for caries, at least through 6 years of age. To develop stable colonization *S. mutans* requires nonshedding tooth surfaces over a period of time. Children generally harbor several *S. mutans* genotypes, with a tendency to form stable colonization by strains transmitted from the mother.[107] Based on epidemiologic evidence from caries prevalence surveys, it is postulated that a second window of infectivity may exist coincident with the emergence of permanent teeth between 6 and 12 years of age.

Streptococcus suis is an emerging zoonotic pathogen found in pigs raised in suboptimal conditions. It affects humans who have direct contact with pigs and pork, with little evidence for person-to-person spread among humans. In three outbreaks in China, in 1998, 1999, and 2005, over 200 people were infected, and 53 died. Nearly three quarters of cases reported have been from China, Thailand, and the Netherlands.[11]

5.3. Geographic Distribution

Streptococcal diseases are of worldwide importance. Group A streptococci appear to be well adapted to humans living in temperate or tropical climates, although differences in the temporal distribution and perhaps the characteristics of disease may vary. In some tropical areas, groups C and G streptococci are more frequently isolated in cases of pharyngitis than are group A strains. As noted above, group B streptococcal carriage is widespread, but infection rates are highest in North America and western Europe, with lowest rates reported from the United Kingdom, northern Europe, and parts of Asia. Pneumococci are the leading cause of bacterial respiratory infection in all parts of the world. In some areas, such as sub-Saharan Africa where seasonal epidemics of meningococcal disease are common, pneumococcal disease remains endemic and accounts for many cases of meningitis throughout the course of the year.

Dental caries occur worldwide. Although caries have been more common in industrialized areas than in developing countries,[150] recent international comparisons have been encouraging, with about 50% of children caries free across 17 countries surveyed.[148] Sweets and sugary drinks continue to play a modest role, but the most important factor now is seen to be parental beliefs and attitudes (the "Brushing Parental Efficacy Factor") that promotes earlier and better supervision of tooth brushing and dental care.[148] Although this has been attributed in part to the higher use of sucrose and refined foodstuffs and social changes associated with urbanization, the major factors remain lack of dental care. The incidence of caries is mitigated to some extent by the availability of artificial fluoridation in dentifrices and water supplies.[152] Caries have always been less common in geographical areas with natural fluoridation.

5.4. Temporal Distribution

Group A streptococcal infections follow characteristic seasonal patterns. In northern parts of the United States streptococcal pharyngitis is typically seen over the winter months, peaking in February or March, while skin infections occur mainly in the summer. Figure 3 illustrates this pattern in surveillance data from private pediatric practices in Rochester, New York, for the 12 years 1977–1988. In the southeastern states there is both a late fall and a late winter peak of respiratory infection, coinciding with the beginning of school in the fall and with increased indoor activity or crowding during colder months. Seasonal variation is less evident in tropical or subtropical areas, although gathering of children at school or other institutions appears to increase the incidence of disease at certain times of the year. Streptococcal skin infections are most common in the rainy season in tropical areas. Such conditions favor the exposure of unprotected skin to the assault of minor trauma and the bites of mosquitoes and other insects. Organisms present on the skin surface, or rarely from the respiratory tract, may be inoculated into damaged skin by itching or scratching.

In temperate climates pneumococcal infections tend to peak in winter months. The disease incidence is not related to the carriage rate, which varies only slightly over the year, but parallels the markedly seasonal rate of acquisition of new strains.[165] In the study of Hodges and MacLeod there was also a strong correlation of pneumococcal lobar pneumonia with the seasonal peaks of influenza virus infection.[164] Present day findings suggest that the association with influenza is rather weak, although a stronger association may be noted for respiratory syncytial virus.[167] In the "meningitis belt" of Africa pneumococcal meningitis epidemics usually occur along with meningococcal epidemic

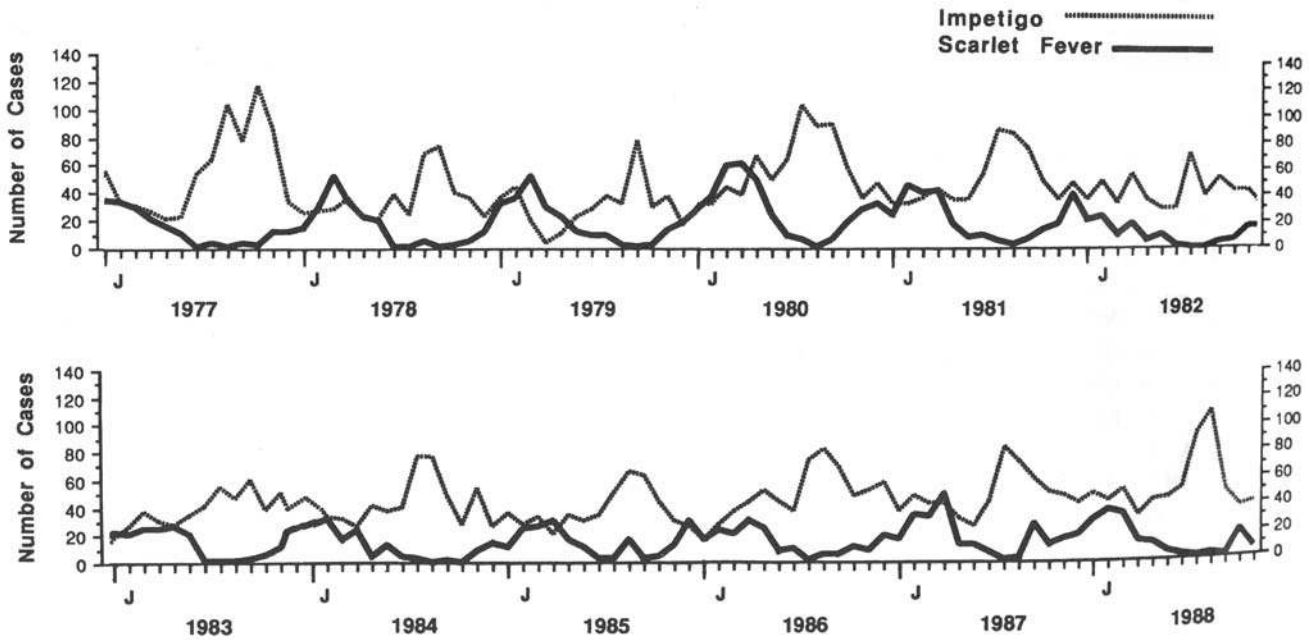


Figure 3. The number of cases of scarlet fever (*solid lines*) and streptococcal impetigo (*dotted lines*) seen monthly in private pediatric practices participating in streptococcal surveillance studies in Rochester, New York, 1977–1988. The data were kindly provided by Caroline Breese Hall, University of Rochester.

season, beginning with the dry weather in February–March. This seasonal pattern is not understood but may involve increase indoor crowding because of the weather and also change in the respiratory mucosa in response to severe drying.[168]

5.5. Age

Group A streptococcal pharyngitis is uncommon in children under 4 years of age. It increases in frequency as children enter school, peaking at 9–12 years of age. These children are the primary source of respiratory infections that occur among families and are thus a source of exposure for parents and other adults in the household. Streptococcal impetigo, in contrast, is chiefly a disease of younger children. It is frequently seen in toddlers and children under 4 and typically reaches a peak incidence at about 6 years of age. Adolescents tend to be subject to milder and often self-limited disease.[122,169]

Group B streptococcal remains a common cause of sepsis and meningitis in neonates and infants up to about 3 months of age.[14] Prematurity is the major risk factor in late-onset disease.[138] Group B streptococci are a common though sometimes disregarded cause of urinary tract infection among adult women, especially during pregnancy, and are an important cause of perinatal and postpartum infection.[162] This has been associated with late abortions and problems of the perinatal period. Rarely does an older child or adult develop a serious infection in the absence of some compromise of normal host defenses. Older adults, however, account for as much as 40% of all group B streptococcal infections. Most common are cases of cellulitis or pressure sores, urinary tract infection, bacteremia, and pneumonia. These are often associated underlying conditions, especially diabetes mellitus, nursing home residence, cardiac disease, and malignancy.[143]

Pneumococcal disease is seen in all age groups, but principally affects the very young and the very old. Among children, 70% of meningitis and bacteremia occur in those under 24 months of age, peaking at 8–12 months of age. The introduction of a 7-valent conjugate vaccine in 2000 resulted in a 60% reduction in invasive disease with the largest decline in infants <12 months of age.[170] The incidence of bacteremic infections increases sharply with age over 55 years, and the case-fatality rate in this group is over 80%. A welcome indirect effect of the 7-valent pediatric vaccine has been the reduction of pneumococcal disease among adults over age 65.[15,171]

Serious infections by enteric and oral streptococci are less subject to the effects of age than to the natural defenses of the host. Low-birth weight babies are increasingly infected by group D and viridans streptococci and

enterococci, perhaps because of the kinds of interventions required for the management of very premature infants or changes in patterns of antibiotic use. Nosocomial infection caused by enterococci spans the range of age, but is most common among older hospitalized adults.[12]

Dental caries is a chronic disease that continues throughout life.[149] Longitudinal studies from diverse localities show that rates of development of new caries are about the same in adults as they are in children, although older adults are more likely to get root caries and gum disease. However, as noted in Section 5.2, there appears to be an age-specific window of acquisition of *S. mutans* at 18–30 months of age that correlates with subsequent risk of caries development.[8]

5.6. Sex

Sex is not a factor in the development of streptococcal disease, except as it relates to pregnancy and to specific genitourinary infections, especially those caused by group B streptococci.[162] These organisms may be transmitted by close, intimate, or sexual contact, but genital colonization is not associated with symptoms in either sex.[172] Colonization may be higher in women during the first half of the menstrual cycle, perhaps because of greater adherence to vaginal epithelial cells at this time. The peptostreptococci are recognized in pelvic inflammatory disease but are rarely encountered (or looked for) elsewhere.

5.7. Race and Genetic Factors

No one is spared from susceptibility to streptococcal infections. Much of the association between race and susceptibility can probably be accounted for extrinsic factors, such as poverty, crowding, and lack of medical care. Nevertheless, there is growing evidence that certain persons within racial groups may have some genetic host predisposition. Studies of histocompatibility leukocyte antigen (HLA) distributions point to increased risk of rheumatic fever for blacks expressing the DR2 phenotype and for whites with the DR4 phenotype.[75] Agammaglobulinemia and IgG subclass deficiencies are associated with recurrent pneumococcal infection. Various complement deficiencies, especially that of the second component (C2), should also be considered in children with repeated pneumococcal disease. Children and adults with sickle cell disease are at increased risk because of functional asplenia, which develops after repeated episodes of splenic infarction. Down's syndrome and cleft palate are associated with increased frequency of otitis media due to pneumococci and other organisms.

5.8. Occupation

Occupation is rarely a factor in the development of group A streptococcal infections. Physicians, nurses, and laboratory workers in close contact with infected patients or their cultures seldom develop disease. Epidemics of skin infections have occasionally appeared in meat packers, and foodborne.[132,173] Group B streptococci are a major cause of mastitis in cattle. Spread is thought to occur mainly via the hands of dairy workers from human or bovine sources. Molecular studies suggest that at least two lineages of strains from bovine mastitis are very closely related to some isolates causing human disease.[174] Consumers of raw infected milk may become colonized with group B streptococci but do not appear to be at risk for disease. Groups A and C streptococcal diseases have occasionally been associated with milk-borne transmission.[132] Group C infections have also been associated with exposure to horses and other animals. Workers in the pork industry occasionally develop meningitis due to *S. suis,* as noted above.[11] *Streptococcus iniea,* a common fish pathogen, has been associated with infections in workers at a fish farm and others handling or cooking fish.[132]

5.9. Other Settings and Predisposing Factors

Family studies of respiratory bacteria have indicated that the group A streptococcus and the pneumococcus enter a family unit most commonly via the school-aged children.[122] These organisms spread rather slowly to other family members, some becoming colonized and very few developing overt infection. Spread is often facilitated by concomitant viral respiratory infections. Socioeconomic factors, crowding, and substandard housing have frequently been cited as contributing to the spread of these organisms and to the development of acute rheumatic fever and bronchopneumonia. Schools, day care centers, nursing homes, and long-term care facilities may experience periodic problems with group A streptococci.[175] Spread of pneumococci generally occurs slowly with little consequence in day care centers, but occasional outbreaks of serious disease have been reported in child care and nursing home settings.[176]

The military has been associated for over a century with increased risk for developing streptococcal disease, especially acute rheumatic fever and pneumonia.[164,177] Our present understanding of the epidemiology of rheumatic fever and methods for antibiotic prophylaxis date from important studies done in the US Armed Forces during and after World War II. Rates of streptococcal pharyngitis are much lower in recruit camps where penicillin prophylaxis was routinely used. In one report, however, antibiotic prophylaxis was not effective until penicillin-allergic recruits,

who continued to harbor streptococci, were given oral erythromycin.[178]

Nosocomial spread of groups A and B streptococci continues to be reported, especially from obstetric and surgical services and newborn nurseries.[142,156] Sources of infection may be other patients or personnel who carry organisms in the nose, throat, skin, vagina, or anus. Enterococcal infections in neonates and surgical patients usually originate from the patient's own flora, under the influence of physical interventions and antibiotic therapy.[12,58,121] Intraabdominal wounds, burns, intravascular catheters, and the urinary tract are the most frequent sites associated with nosocomial enterococcal bacteremia. Pneumococci are important but infrequent causes of nosocomial outbreaks.[176]

Predisposing host factors may be genetic or acquired, such as sickle cell disease, immunodeficiency, or splenectomy. Splenectomized patients have a 50- to 200-fold greater risk of serious bacteremic infection.[179] Surgical repair, rather than removal, of a traumatized spleen is frequently possible and always preferred to removal. Overall, about a fourth of children with pneumococcal bacteremia or meningitis have some underlying condition or host defense abnormality, and risk for these children persists well beyond 24 months of age. Patients with human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS) are at particularly high risk of bacteremic pneumococcal infection.[180] Intravenous drug abuse, also a known AIDS risk factor, is associated with an increase in group G streptococcal infection.

6. Mechanisms and Routes of Transmission

The transmission of streptococci, introduced in Section 5.2, depends on factors such as the usual ecologic niche of the species, its occurrence as part of the normal flora, and the ease with which it may be carried by hands, in secretions, or possibly in droplets. The group A streptococci are transmitted principally by direct contact. Contagiousness is greatest during acute respiratory infection, whereas the chronic carrier is a relatively low risk as an infectious source. This probably reflects the fact that development of infection requires a fairly large inoculum. About 100 organisms are required to infect adult volunteers by directly inoculating the tonsils and pharynx with organisms on a cotton swab. Although streptococci may occur in droplets and survive in dust, an individual is unlikely to acquire a large enough inoculum to become infected from these sources. Streptococci recovered from contaminated blankets and dust may also be less infective. Direct transmission of respiratory secretions may occur via hands, person-to-person contact such as kissing, or projection of large droplets during coughing or sneezing. Treatment of infected persons eliminates the risk of spread within 24 h, permitting resumption of work or school activities as soon as constitutional symptoms have abated.[181]

Pneumococci are thought to be transmitted by similar routes.[182] Coughing is a more regular feature of pneumonia than it is of other pneumococcal infections or of streptococcal pharyngitis. Transmission appears to be favored by symptoms of cold and coryza. Dust particles may harbor pneumococci but do not play a significant role in transmission.[164] Direct spread of secretions containing viable organisms is the most likely source. Covering one's mouth with a hand may deflect a cough or sneeze, but the hand may then transmit fresh secretions.

Large milk-associated epidemics of group A streptococcal disease such as occurred in early in the last century have now been essentially eliminated by the widespread adoption of pasteurization.[132] Small outbreaks of group A and C infections continue to be seen with contaminated prepared foods, such as egg salad, or with cheese and dairy products from cows or goats.[131,132] Despite the prevalence of group B streptococci in bovine mastitis, milk-borne group B infections have not been reported. However, some virulent group B strains appear to be genetically related to lineages of bovine origin.[174]

Skin infections due to group A streptococci are often caused by serotypes different from those associated with throat infections (see Table 3). Similarly, certain corresponding *emm* types are markers for strains with a strong preference for skin infection.[38,75] Streptococci may occur on normal skin but do not cause infection unless there is a break in the cutaneous epithelium. Minor injuries such as scratches, abrasions, cuts, or insect bites may become points of entry. Mosquito bites are an especially common site of inoculation, where organisms are rubbed into the wound during itching. In Trinidad, streptococcal impetigo may be transmitted by flies of the genus *Hippelates*, which feed on open skin sores. Streptococci from the nasopharynx may be transmitted to skin sites, but it is more common for the skin to be colonized first and the throat later. Classical erysipelas, in contrast, is thought to be transmitted via the respiratory tract, either from the patient or from a caretaker. Erysipelas is occasionally seen following superinfection of chicken pox lesions.

The group B streptococci causing early-onset perinatal infections are those carried by the mother in the lower gastrointestinal and genitourinary tracts. Organisms may enter the amniotic cavity following rupture of amniotic membranes, but the fetus may become infected with membranes intact. Although rupture of membranes for greater than 12 h is a recognized risk factor for ascending infection,

the duration of labor is also important, perhaps because of the entry of organisms via microscopic defects in the membranes. Among twins, who have a higher rate of infection than singletons, it is usually the first twin who is closer to the source of infection and at greater risk. Once the streptococci have entered the amniotic fluid, they proliferate rapidly and are aspirated or swallowed by the fetus. Late-onset infections may have a maternal source in about half of cases, with colonization occurring at delivery.[26,138] Other infants may acquire streptococci later from persons outside the immediate family, presumably via the oral or respiratory route. Late-onset and recurrent infections have also been transmitted by mother's milk in cases with mastitis, asymptomatic colonization, or banked breast milk.[183]

Oral streptococci are part of the normal flora and are acquired early in life from the mother and family members.[7,8,107] Enteric streptococci generally cause opportunistic infection only after perturbations of normal defenses. Nosocomial infections are usually due to a patient's own flora. Transient bacteremia may occur with dental manipulation, transurethral prostate resection, or gynecologic and gastrointestinal surgery. Patients with known history of valvular heart disease or artificial heart valves are at higher risk for developing endocarditis due to otherwise "benign" streptococcal species. In such cases a prophylactic antibiotic is administered just prior to the dental or surgical treatment (see Section 9.2). Enterococcal infections may be similarly opportunistic or nosocomial in origin.[12]

7. Pathogenesis and Immunity

7.1. Pathogenesis

The development of infection involves a multitude of interrelated bacterial and host factors that vary enormously among streptococcal species. The biologic characteristics associated with virulence among various streptococci are summarized in Section 4; certain host factors and modes of transmission are noted in Sections 5.9 and 6. The pathogenic process is not clearly understood for any streptococcal disease, but it may be useful to consider three general stages.

First, organisms are acquired by the host and either succeed in colonizing their preferred sites or are eliminated by host defenses at the epithelial surface. For many streptococcal species the specific mechanisms of adherence are known.[75,78,92,184] Adherence may be important in the establishment of colonization but is not necessarily a property that distinguishes virulent from commensal species or strains. To ward off bacterial invasion the host has epithelial barriers, mucus layers, and secretions containing enzymes and antibodies.

The second stage begins when organisms succeed in breaching local defenses and enter the epithelial and subepithelial tissues, or in the case of *S. mutans* get into and through the tooth enamel.[75,92,184] Most of the acute pathologic effects of group A streptococci occur at this stage by causing pharyngitis or skin infection, with invasion of the submucosal or subepidermal layers. This is frequently accompanied by lymphadenitis but rarely progresses to bacteremia. In pneumococcal pneumonia and in otitis media the initial insult is not a direct breach of epithelium but rather the invasion of a normally sterile compartment; symptoms of disease ensue as organisms multiply and induce inflammation in surrounding tissue. The host responds with nonspecific secretory and serum forces, including the release of vasoactive and chemotactic mediators, activation of the alternative complement pathway, and with the mobilization of neutrophils and tissue macrophages. Eventually, specific secretory and serum antibodies develop and contribute to resolution of the disease.

The third stage is systemic infection, in which organisms multiply in blood and tissues. Severe streptococcal infections, including streptococcal toxic shock, depend upon the organism's ability to evade host defenses until it establishes a sufficient mass of growth to cause systemic manifestations. The M proteins protect against phagocytosis and also down-regulate complement activation on the bacterial surface.[73] C-5 peptidase is an enzyme that inactivates complement C5a and decreases chemotaxis and secondary inflammatory responses.[87] Spread of infection within the tissues may be facilitated by the action of hyaluronidase and proteinases. Shock is probably mediated by tumor necrosis factor-α (TNFα), interleukin (IL)-1β, and IL-6, all of which may be induced by pyrogenic toxins (SPE), streptolysin O, and cell wall breakdown products. Further alterations in host physiology occur by cytokine activation and by direct and indirect effects on hemodynamics, metabolism, and the function of individual organs.[4,185,186]

Similar mechanisms are involved in acute group B streptococcal sepsis.[92,187] Early-onset infections usually begin in utero, and it is suspected that the organisms gain access to the circulation by breaching the gastrointestinal or pulmonary epithelial barriers. The organisms may be actively taken up by pulmonary alveolar cells acting as nonprofessional phagocytes, which are incapable of killing the organisms preventing tissue invasion. Alternatively, the organisms can cross the epithelial barriers by transient openings in the cell junctions.[188] Group B streptococci also have mechanisms to evade the inflammatory responses of the host, including the antiphagocytic properties of the polysaccharide capsules,

a C5a inactivating enzyme similar to that of group A streptococci, and the c protein, which binds IgA in a nonimmune fashion. The early manifestations of severe disease depend upon the inflammatory responses mediated by both specific and innate host defense factors. Group B streptococcal surface structures and breakdown products, especially cell wall peptidoglycan, interact directly with macrophages and monocytes promoting release of proinflammatory cytokines. Cell wall activates C3 complement via the alternative pathway, and both peptidoglycan and lipoteichoic acid (LTA) interact with components of the Toll-like receptor (TLR) system, which in turn promotes cytokine responses. Tissue damage due to hemolysins and other toxins further the inflammatory response. While the initial inflammatory response is often minimal in infants, despite the presence of bacteria in the lung or other tissue, localized, overexuberant responses in the central nervous system appear to play a major role in disturbing the blood–brain barrier, opening the way to development of meningitis.[92,187]

The most severe infections, particularly streptococcal toxic shock syndrome, have all the elements of invasive disease, and in addition have the effects of streptococcal superantigens.[75,89,185,186] These include streptococcal pyogenic exotoxins (SPE) A, B, C, F (mitogenic factor), G, H, J, and are much like the staphylococcal toxic shock toxin (TSST-1) and staphylococcal exotoxins SE A–E and H. The absence of neutralizing antibodies to these toxins is associated with increased risk of severe disease.[189] Superantigens are a family of protein toxins that activate the immune system by binding directly to both the MHC class II molecules of antigen-presenting cells and the Vβ T-cell receptor, causing nonspecific activation of up to 30% of the entire T-cell population. This in turn causes massive release of cytokines, including TNFα, interferon-γ, IL-2, and IL-6. More T and B cells are recruited to the site of infection, resulting in further release of IL-1, TNFα, and other mediators. The triggering of an overexuberant host response is thought to play a large role in hypotension, disseminated coagulopathy, and multiorgan failure seen in toxic shock syndrome.

The pathogenesis of the nonsuppurative sequelae of group A infections has yet to be fully elucidated. Examples of the cross-reactivity between M proteins and components of heart, brain, and connective tissue have now been convincingly confirmed and add weight to the long-held hypotheses of autoimmune mechanisms for rheumatic valvular disease, Sydenham's chorea, and skin manifestations.[75] Although multiple complex factors appear to be involved in disease process, these and other studies have identified M protein epitopes that cross-react with myosin, DNA, and α-helically coiled proteins such as tropomyosin, actin, and keratin. Antigenic mimicry between cell wall carbohydrate moieties and cytokeratin may eventually help explain the occurrence of erythema marginatum, subcutaneous nodules, rheumatic arthritis, and perhaps streptococcal-associated guttate psoriasis. The diverse manifestations of acute rheumatic fever might be explained in part by the specificity and titer of antibodies cross-reactive with different tissue components. In a similar fashion, acute glomerulonephritis may involve the development of antibodies that cross-react with basement membrane collagen and laminin.[75] The cationic charge of the antigens may also influence their affinity for the glomerular basement membrane and the nature of immune complexes formed. Another feature of nephritogenic streptococci is the presence of a nephritis strain-associated protein.[85] This distinctive low-molecular-weight streptokinase is a plasminogen activator and could induce proliferation of cells, release of inflammatory products, and activate complement, thus contributing to known pathologic features of the disease.

The pathogenesis of dental diseases has been described as "examples of ecological catastrophes."[190] Caries development involves dynamic relationships among dental plaque organisms, dietary carbohydrates, and mucosal host defenses. Fermentable carbohydrates, especially sucrose, favor the adherence of mutans streptococci to the tooth surface by several different mechanisms, including glucan-binding and salivary-binding proteins. *Streptococcus mutans*, together with *Streptococcus sobrinis,* lactobacilli, and other bacteria form biofilms on the tooth surface. The biofilms have an extracellular water-insoluble carbohydrate matrix which helps stabilize and protect the bacterial colonies and concentrate acids from fermentation, which in turn lower the pH, and aid in demineralization of tooth enamel. This process is countered by the host by the buffering capacity of saliva and defensive components, such as lysozyme, lactoferrin, and secretory IgA. Specific antibodies to *S. mutans* surface proteins can inhibit sucrose-dependent adherence and interfere with biofilm formation.[107,184]

7.2. Immunity

Specific immunity to streptococcal infections is thought to depend on the development of antibodies to capsular and other surface determinants. Antibody assays for various streptococcal antigens have been noted in Section 3.4.2, and current developments in streptococcal vaccines are further discussed in Section 9.6. Although most of our knowledge of the immune response relates to the occurrence of antibodies in the serum, recent studies of mucosal immunity suggest that the first line of host defenses are at the local level. The precise role of secretory antibodies in preventing, modifying, or eradicating bacterial colonization is not presently understood. Conversely, the effects of bacterial antigens at

respiratory and gastrointestinal sites are poorly understood in regard to the systemic antibody response and the development of autoantibodies. In some cases it is likely that the organisms have developed a form of mimicry to confuse or evade the immune response of the host. Many streptococci have antigens that cross-react with other organisms, common foodstuffs, or components of the host's own tissue. Antibodies undoubtedly have functions other than protection against disease. They are known to be important in the recovery from established infection and probably function in the removal of antigens from local and systemic sites.

Assays for group A streptococcal antibodies (Table 5) are used clinically to determine if a patient has had a recent infection.[72] Although humans normally make antibodies to streptolysin O and other extracellular components, only antibodies to M proteins are associated with protection against subsequent disease.[73,75,185] Serum IgG antibodies to M proteins arise in response to infection or immunization and can initiate bactericidal activity in whole blood opsonophagocytic assays. These antibodies, however, are not as efficient as secretory IgA antibodies in protecting mice against infection via the intranasal route. Intranasal immunization of volunteers with purified M protein resulted in greater resistance to colonization with live streptococci than did subcutaneous immunization, and serum antibody response was not a reliable predictor of resistance to pharyngitis. At present, group A vaccines remain experimental, but it appears that local immune mechanisms may be more efficiently utilized and less likely to result in systemic side effects.

Antibodies to the capsular polysaccharides of group B streptococci are thought to mediate protection against systemic invasion. This concept is based on the finding that few infants with group B streptococcal disease have antibodies to the offending type, and that antibodies are protective against bacterial challenge in animal models.[191] Naturally occurring antibodies in mothers are often of the IgM class and do not readily cross the placenta, and less IgG antibody is transferred to the premature than to the term fetus. Protective levels of IgG seem to vary with serotype, and protection increases as fetuses receive more maternal IgG at > 34 weeks gestation. In a prospective study of infection levels have been empirically estimated to be ≥ 5 μg/ml for type Ia and ≥ 10 μg/ml for type III.[192,193] Despite the presumed importance of antibodies, it should be pointed out that the prevalence of group B streptococcal antibodies among pregnant women is generally low, with only 7–23% sera having >2 μg/ml against types Ia, Ib, II, III, and V in a defined population.[194] Thus, the majority of infants are "antibody deficient," whether colonized or not, yet only a small number develop disease, even when exposed to infected amniotic fluid in utero.[195] The presence of mucosal antibodies may modify colonization, but so far a clearly defined role for secretory immunity is not apparent.

Most of our knowledge of pneumococcal immunity comes from studies of purified polysaccharide vaccines. Adults respond well to most of the antigens, but infants respond poorly. Conjugate vaccines improve the immunogenicity of vaccines by coupling the polysaccharides to protein carriers to elicit T-helper cell involvement in the immune response. Although an IgG level of >0.2 mg/ml has been used as a marker of vaccine response, there is no consensus as to what constitutes a protective serum antibody level.[196]

8. Patterns of Host Response

8.1. Clinical Features

The manifestations of streptococcal diseases are remarkably diverse. Their clinical presentation, diagnosis, and therapy are discussed in detail in many of the medical, pediatric, and infectious disease textbooks. The most common and most important diseases caused by the major streptococcal group are listed in Table 6. Diseases associated with other streptococci are noted in Tables 1 and 2.

The typical form of group A streptococcal respiratory disease in children and adults is acute exudative pharyngitis. The onset is abrupt, with fever, chills, a sore throat with pain on swallowing, malaise, headache, often with abdominal pain, nausea, or vomiting. On physical examination, the pharyngeal mucosae are erythematous, edematous, and streaked with a purulent nonadherent exudate over the tonsils or posterior pharynx; petechiae may be present. The anterior cervical lymph nodes may be enlarged and are usually acutely tender. In young children the findings may be less specific, often with fever and lymphadenitis but relatively little inflammation of the upper respiratory mucosae. Mild or subclinical infection may occur at any age and may be missed. This is of concern because up to half of patients developing rheumatic fever do not give a clear history of an antecedent sore throat. With scarlet fever the pharyngitis is accompanied within about 24 h by a fine red exanthem beginning on the trunk and intertriginous areas, later spreading to the extremities. The face is usually spared, but the tongue may be inflamed with a "strawberry" appearance. Careful auscultation of the heart should be part of the physical examination on all patients with acute streptococcal sore throat or scarlet fever.

Streptococcal impetigo may appear as single lesions of the epidermis, frequently on the lower extremities, spreading to other areas as new insect bites or breaks in the

Table 6. Diseases Associated with Major Streptococcal Groups

Group A streptococci	Group B streptococci	Pneumococci	Enterococci
Most common	Early-onset neonatal disease	Infants and children	Nosocomial
Pharyngitis/tonsillitis	Undifferentiated sepsis	Otitis media	Bacteremia
Scarlet fever	Meningitis	Conjunctivitis	Intra-abdominal infection
Impetigo/pyoderma	Pneumonia	Pneumonia	Surgical wounds
Cellulitis	Bacteremia	Bacteremia	Burn wounds
Less common	Maternal infections	Meningitis	Vascular catheter
Toxic shock syndrome (Table 7)	Amnionitis	Epiglottitis	Urinary tract infection
Peritonsillar abscess	Endometritis	Adults	Postpartum infections
Mastoiditis	Urinary tract infection	Sinusitis	Community acquired
Sinusitis	Bacteremia/sepsis	Pneumonia	Urinary tract infection
Otitis media	Septic abortion	Pleural empyema	Endocarditis
Erysipelas	Bacteremia	Endocarditis	Biliary infection
Pneumonia/empyema	Late-onset neonatal disease	Meningitis	Pelvic infection
Puerperal sepsis	Meningitis	Endocarditis	
Meningitis	Bacteremia/sepsis		
Endocarditis	Bone and joint infection		
Proctitis	Skin and soft tissue infection		
Vulvovaginitis	Otitis media		
Nonsuppurative sequelae	Omphalitis		
Acute rheumatic fever			
Acute glomerulonephritis			

skin become infected. Early lesions are pustular and rapidly develop into mature lesions about 1 cm in diameter with a characteristic honey-like crust. Lesions may become concomitantly infected by staphylococci, which may be resistant to penicillin therapy. Erysipelas is a serious superficial soft tissue infection that extends into the subcutaneous lymphatics and appears with a rapidly advancing well-demarcated border. Erysipelas often involves the face, a surgical wound, an umbilical stump, or a chicken pox lesion. The patient is febrile, toxic, and may be bacteremic. Necrotizing fasciitis is a deep-seated infection of subcutaneous tissue that destroys fascia and fat but may also destroy skin and muscle. Patients may be diabetic or otherwise debilitated. Recently reported cases have occurred mainly in previously healthy individuals who had trivial or inapparent trauma at the affected site. A hallmark, though nonspecific, is the rapid progression from tenderness to severe pain at the site of infection. Infections may become gangrenous and require surgical debridement or fasciotomy.

Streptococcal toxic shock has been observed with varying frequency in Europe and North America during the past decade or more.[4,13,20] Persons may be affected at all ages, usually without predisposing or underlying diseases. Most cases involve a skin or soft tissue focus of infection or pneumonia (as in the case of puppeteer Jim Henson), and most patients are bacteremic. In children a large proportion of cases are associated with infected chicken pox lesions, but this has almost disappeared as a risk factor since the routine use of varicella vaccine has been implemented. Criteria for diagnosis of streptococcal toxic shock are given in Table 7.[197] This syndrome differs from other serious streptococcal infections, and to some extent from staphylococcal toxic shock, by the rapid development of hypotension and multiorgan failure early in the course of infection. Streptococcal toxic shock is rarely associated with sunburn-type rash; however, such a rash is common in staphylococcal toxic shock syndrome.

The nonsuppurative sequelae of group A infections may present acutely or insidiously. Acute rheumatic fever varies greatly in its manifestations. The diagnosis was made with the guidance of the Jones Criteria (updated in 1992),[198] described in Table 8. The onset is typically abrupt, with fever and polyarthritis. Myocarditis or valvulitis, most commonly involving the mitral valve, occurs in about half of patients suffering their first attack. Carditis may be the only major manifestation in some patients and may develop insidiously, presenting as heart failure without any clear history of prior rheumatic fever or obvious streptococcal infection.[3,198] Because of difficulties in making the diagnosis, other conditions, such as collagen-vascular diseases and infective endocarditis, must be considered whenever acute rheumatic fever is suspected. Acute glomerulonephritis presents fewer diagnostic problems. A recent skin or throat infection is usually evident by examination, history, culture, or antibody tests. The urine is dark, containing many red blood cells and casts. The patient usually has edema and

Table 7. Proposed Criteria for Diagnosis of Streptococcal Toxic Shock[a]

I. The isolation of group A streptococci
  A. From a normally sterile site, such as blood, CSF, surgical wound, pleural fluid, etc.
  B. From a nonsterile site, such as throat, open wound, or superficial skin lesion, or vagina
And
II. Clinical signs of severity
  A. Hypotension; systolic blood pressure ≤90 mmHg in adults or <5th percentile for age in children
  And
  B. Two or more of the following:
    1. Renal impairment evidenced by elevated creatinine ≥2 mg/dl (177 μmol/l) or twice the upper limit of normal for age; or, for patients with preexisting renal disease, ≥2 times baseline level
    2. Coagulopathy: thrombocytopenia (<100,000/mm^3) or disseminated intravascular coagulation, defined by prolonged clotting time, low fibrinogen, and presence of fibrin degradation products
    3. Liver involvement: elevated liver enzymes or total bilirubin ≥twice the upper limit of normal for age; or, for patients with preexisting liver disease, ≥2 times baseline level
    4. "Adult" (acute) respiratory distress syndrome (ARDS), defined by onset of diffuse pulmonary infiltrates and hypoxemia in absence of cardiac failure; or evidence of diffuse capillary leak manifested by acute generalized edema, or pleural or peritoneal effusions with hypoalbuminemia
    5. Generalized erythematous macular rash, which may desquamate
    6. Soft tissue necrosis including necrotizing fasciitis, myositis, or gangrene
A *definite* case is defined by isolation of group A streptococci from a normally sterile site (IA) plus hypotension (IIA) and supporting clinical signs of severity (IIB). A case is defined as *probable* if the culture was from a nonsterile site (IB) and it fulfills the clinical signs of severity (IIA + B), when no other etiology for the illness can be identified

[a]Adapted from The Working Group on Severe Streptococcal Infections.(196)

Table 8. Jones Criteria (Revised) for Guidance in the Diagnosis of Acute Rheumatic Fever, as Recommended by the American Heart Association

Major manifestations	Minor manifestations
Carditis	Clinical
Polyarthritis	Fever
Chorea	Arthralgia
Erythema marginatum	Previous rheumatic fever or rheumatic heart disease
Subcutaneous nodules	
	Laboratory
	Acute phase reactions: abnormal erythrocyte sedimentation rate, C-reactive protein, or leukocytosis
	Prolonged P–R interval

Supporting evidence of streptococcal infection:
  Positive throat culture for group A streptococcus or positive rapid streptococcal antigen test
  Increased or rising antistreptolysin O or other streptococcal antibody
The presence of two major, or one major and two minor, manifestations *plus* evidence of a preceding streptococcal infection indicates a high probability of rheumatic fever
Manifestations with a long latent period, such as chorea and late-onset carditis, are exempt from the latter requirement. The WHO Study Group recommends that the following groups be considered separately and exempted from the Jones criteria: "pure" chorea, insidious or late-onset carditis, and rheumatic recurrence(197)

elevated blood pressure; the blood urea nitrogen and creatinine are usually elevated and the C3 complement level is low. With appropriate acute care, nearly all patients have a complete recovery.

Group B streptococcal disease in the neonate usually begins in utero with nonspecific symptoms appearing within the first few hours of birth.(26,163) Unexplained apnea, respiratory distress, temperature instability, or poor feeding may be the only clues to early-onset disease. Infection usually takes the form of undifferentiated sepsis with, or more often without, meningitis. The disease is well advanced by the time hypoxia, cyanosis, acidosis, or vascular collapse becomes obvious. Lung involvement is frequent but the chest X-ray is typical of hyaline membrane disease more often than of discrete pneumonia. Similarly, there may be little or no CSF pleocytosis even when bacteria are recovered from the

spinal fluid. In general, the inflammatory response parallels the maturity of the infant, the specificity of clinical signs and symptoms, and to some extent the prognosis. Late-onset infections, occurring beyond the immediate newborn period, often tend to be more localized and have a better outcome. Meningitis is common, but sepsis without meningitis is frequently seen. Bacteremia or meningitis may be associated with an infective focus, such as omphalitis, otitis media, or osteomyelitis. The long-term outcome of infants who survive group B streptococcal meningitis is generally good. Very occasionally, neonates develop similar disease due to group G streptococci or pneumococci. Maternal group B streptococcal disease may occur during gestation, notably urinary tract infections and septic abortion. Infection around the time of delivery, especially amnionitis, is a threat to both fetus and mother. Endometritis and bacteremia are the most frequent postpartum infections. Endocarditis is an unusual complication of perinatal infection or septic abortion.[162]

Group B streptococcal disease also occurs in adult men and nonpregnant women. In a recent survey of invasive group B infections metropolitan Atlanta hospitals clinical diagnoses included skin, soft tissue, or bone infections (36%), bacteremia without focus (30%), urosepsis (14%), pneumonia (9%), and peritonitis (7%).[142] Another excellent clinical series, which also included data on group A, C, F, and G streptococcal infections, found that the case mortality rate was 31% among adults with group B streptococcal bacteremia.[199] Two-third of all patients were over 50 years of age, 22% had primary bacteremia, 25% had underlying nonhematologic malignancies, and 19% had diabetes mellitus. At least 50% of the mortality due to group B streptococci now occurs in the age group >65 years, attributed mainly to pneumonia and bacteremia without an identified focus.[143]

There is good evidence that group C and G streptococci are a cause of endemic pharyngitis in adults in open populations.[200,201] Of the several streptococci falling into serologic group C, it appears that large-colony *Streptococcus equisimilis* is more likely to be associated with clinical disease than the tiny-colony *S. anginosis* ("*milleri*") group. The presence of these organisms must be determined by throat culture, because the rapid *Streptococcus* antigen detection kits are specific for group A streptococci.

9. Control and Prevention

9.1. General Preventive Measures

Personal hygiene, adequate nutrition and housing, health education, and access to medical care are all important factors in prevention of streptococcal diseases. Inspection of food and milk production and proper pasteurization of dairy products are taken for granted in most developed countries but remain problems in certain areas. Strict asepsis is required in surgical and obstetric procedures. Simple infection control measures, especially handwashing, must be continually encouraged, and attention must be paid to the health of hospital employees working in patient areas.

9.2. Antibiotics in Treatment and Prevention of Group A Streptococcal Disease

Primary prevention of acute rheumatic fever consists of identifying and treating persons with acute streptococcal pharyngitis.[30] Antibiotic therapy is aimed at eradicating streptococci from the respiratory tract. Shulman et al. have made the case over and again that penicillin remains the drug of choice for treatment of streptococcal pharyngitis.[110,202] Penicillin may be given as a single intramuscular injection of ("long-acting") benzathine penicillin or as a 10-day course of oral phenoxymethyl penicillin. Other oral penicillins, especially amoxicillin, are also effective but are more expensive and offer no advantage other than palatability. The older oral cephalosporins (cephalexin, cefaclor, cephradine, cefadroxil) are effective in a number of conventional 10-day dose regimens. Several newer cephalosporins (cefprozil, cefpodoxime, loracarbef) are equally effective; 5-day dose regimens are currently under investigation but will not be recommended without further evaluation. Erythromycin is a well-established alternative drug for penicillin-allergic patients, except in some areas where resistance may be a problem. Clindamycin is effective but has a small potential risk of pseudomembranous enterocolitis. Tetracyclines, sulfonamides, and chloramphenicol are not effective.

Spread of streptococci within populations has been an effective means of reducing exposure in households and institutional settings. Mass prophylaxis with benzathine penicillin has been used in some epidemic situations but is now chiefly confined to certain military populations[157] (see Section 5.9). Eradication of pharyngeal carriage of group A streptococci using oral antibiotic regimens has been attempted with varying degrees of success. Effective regimens include amoxicillin/clavulanate (Augmentin®) or clindamycin orally for 10 days.[203,204] Patients with symptomatic streptococcal sore throat may remain infectious for 24 h after the onset of therapy and should not return to work, school, or day care before completing a full 24 h of antibiotic therapy.[181]

Secondary prevention consists of the regular administration of antibiotic to persons who have had rheumatic fever in order to prevent subsequent group A infections that could

trigger recurrent attacks or exacerbate existing rheumatic heart disease.[30] Secondary prophylaxis is cost-effective, reduces the risk of recurrence, and in many patients allows for healing of valvular damage occurring in the initial attack. Long-acting intramuscular benzathine penicillin is usually given at intervals of 2–4 weeks, depending on local prevalence of rheumatic fever. Although dosing every 3 weeks may not provide adequate penicillin levels through the third week, this regime appears to be adequate in most situations and is often recommended.[205,206] Alternatively, oral penicillin may be given daily, but lack of compliance is a major problem. Patients unable to take penicillin may be given sulfadiazine or erythromycin. The duration of secondary prophylaxis is not certain but must be tailored to the individual. Patients with valvular rheumatic heart disease are given prolonged, even lifelong, prophylaxis. Those without cardiac involvement should have prophylaxis for at least 5 years and at least through age 18. Prophylaxis may be safely discontinued in young adults without carditis, provided that they have adequate medical follow-up and prompt culture and treatment of pharyngitis episodes.[207] Patients with rheumatic carditis as part of their initial attack are at greater risk for more serious carditis recurrences and should be given prophylaxis well into adulthood and perhaps for life.

Patients who have had isolated chorea as the only manifestation of rheumatic fever appear to be at much less risk for carditis during subsequent recurrences. Most authorities recommend that these patients receive prophylaxis until age 21 or for at least 5 years, whichever is longer. Some patients have a syndrome of poststreptococcal reactive arthritis without fulfilling Jones' criteria. A few such patients have had silent or delayed mitral insufficiency and later developed acute rheumatic fever.[208] It is therefore generally recommended that patients with poststreptococcal reactive arthritis receive prophylaxis for at least 1 year at which point it may be discontinued if no evidence of carditis develops.[30]

The prevention of acute glomerulonephritis by antibiotic therapy has not been convincingly demonstrated, and prophylaxis may not be practical in most patient populations. Prompt treatment of minor skin trauma and insect bites with topical antibiotic ointment has been shown to reduce the occurrence of streptococcal skin infections and could reduce the risk of subsequent nephritis.[209,210]

Although it is well known that group A streptococci can spread from infected patients to close contacts, available evidence does not support the routine administration of prophylactic antibiotics to close or household contacts of patients with streptococcal toxic shock or necrotizing fasciitis.[211]

9.3. Surgical Approaches to Recurrent Group A Streptococcal Disease

Tonsillectomy or tonsillectomy with adenoidectomy are frequently performed because of recurrent throat infections. Although children who have surgery experience fewer throat infections over the subsequent 2 years, the difference in rates compared to controls is not impressive. These findings were confirmed by Paradise et al. in a carefully controlled study with stringent entry criteria.[212] Surgical intervention was beneficial for severely affected children over at least 2 years following surgery. Nevertheless, a substantial proportion of those managed nonsurgically had relatively little throat infection during the period of study, and the actual reduction in group A streptococcal infection rates was small. Their results appeared to "justify but by no means to mandate the performance of tonsillectomy" in carefully selected children. Treatment should be individualized, and should be considered only for severely affected children. More clearly defined indications for surgical intervention include patients with peritonsillar abscess or severe obstructive symptoms.

9.4. Antibiotics in Treatment and Prevention of Endocarditis

Several national groups periodically issue detailed guideline for management of infective endocarditis.[108,213,214] The major organisms include viridans streptococci and *S. bovis*, *Abiotriphia* and *Granulicatella, S. pneumonia*, group A, B, C, and G streptococci, and *Enterococcus*. Dosage and duration of antibiotic therapy depend upon whether the offending organism is highly susceptible or relatively resistant to penicillin. Enterococci vary in susceptibility to penicillin and gentamicin, and some strains, especially among *E. faecium*, are resistant to vancomycin.

Guidelines for prevention of infective endocarditis have recently been revised by British and American cardiology, dental, and infectious disease organizations.[215,216] Whereas it used to be commonplace to administer antibiotics prior to dental procedures for patients with a wide range of heart valve disorders, now only few limited diagnoses are considered for prophylaxis. These include patients with prosthetic heart valves, patients with a prior history of endocarditis, and those with unrepaired congenital heart disease and those with residual defects, prosthetic materials or devices that inhibit endothelialization. It has been concluded that bacteremia resulting from daily activities is much more likely to cause endocarditis than bacteremia associated with dental procedures. Only an extremely small number of cases would be prevented even if prophylaxis were 100% effective.

Scientific data demonstrating the efficacy of prophylaxis is lacking, except for conditions associated with highest risks for adverse outcomes.

9.5. Intrapartum Chemoprophylaxis Against Group B Streptococcal Disease

The rates of early-onset group B streptococcal invasive disease have declined significantly following the implementation of various programs of maternal screening and administration of intrapartum antibiotics, as described in Section 5.1. The original 1996 guidelines, a compromise that used either maternal culture screening or maternal risk factors, resulted in a 65% decrease in early-onset cases. Subsequent population-based surveillance of the two different approaches found that culture-based programs were at least 50% more effective than risk-based programs.[134] This prompted a revision of the guidelines in 2002 by the CDC in cooperation with the American College of Obstetricians and Gynecologist (ACOG) and the American Academy of Pediatrics (AAP).[28] The new guidelines, outlined in Figure 4, is based on results of combined vaginal and rectal culture performed on women at 35–37 weeks of gestation. Further surveillance during 2002–2004 has confirmed that the rates of early-onset disease have declined an additional 31% since implementation of the new guidelines.[14] Several European countries and Australia have instituted similar guidelines, and Dutch investigators have recommended a shift from risk-based to screening-based programs.[136]

The 2002 guidelines call for using penicillin G as the first choice for intrapartum prophylaxis, with ampicillin or cefazolin as alternatives.[28] A potential problem with antibiotic use in this setting is the possibility of selecting organisms with increased antibiotic resistance. So far no group B streptococci have become penicillin resistant. There is, however, some increased frequency of resistance to clindamycin, which is used as an alternative for penicillin-allergic patients.[27] Resistance to ampicillin among nontarget organisms, such as *Escherichia coli*, parallels rates in other community-acquired infections and does not appear to be related directly to intrapartum prophylaxis. Nevertheless, the resistance patterns of other organisms causing neonatal sepsis should receive continued attention and surveillance.[28]

Prevention of neonatal infections by vaginal chlorhexidine disinfection during labor has also been investigated.[217] A reduction in transmission of group B streptococci from mother to infant has been demonstrated, but numbers have been too small to assess an effect of prevention of infections.[218] This approach might reduce the number of infection that develop as a result to colonization of infants during delivery, but as with intrapartum antibiotics, it should not be expected to affect infections that are already established in utero.

9.6. Passive Immune Prophylaxis Against Streptococcal Infections

The administration of gamma globulin has been employed for prevention of various bacterial infections in immunodeficient patients. The spectrum of host defense abnormalities that may benefit from this approach now includes patients with hypogammaglobulinemias, IgG subclass deficiencies, malignancy and immunosuppression, severe burns, bone marrow transplantation, and HIV infection.[219] Although passive immunotherapy was the only efficacious treatment for pneumococcal pneumonia in the years prior to the advent of antibiotics, no immunoglobulin products are in current use as adjunctive treatment for streptococcal infections.

9.7. Immunization Against Streptococcal Infections

A vaccine against rheumatic fever has been the holy grail of many investigators over the years. As reviewed by Bisno et al., current research involves two broad categories of vaccines: those based on type-specific M protein components and others containing virulence determinants that are common to all or many serotypes.[220] The M protein molecule is a coiled coil with three main antigenic regions: a nonhelical amino-terminus conferring type specificity and susceptible to antibody; the second area consists of B repeat subunits that cross-react with human tissue components; a third C repeat region has epitopes common to many M proteins; finally, the carboxy terminus ends with a cell wall spanning anchor region.[73] A multivalent recombinant M-type vaccine has now been constructed using the amino-terminal peptides of 26 of the clinically most important M types.[221] This vaccine has been tested for safety and immunogenicity in humans, and it elicits opsonizing antibodies to the respective M types. The vaccine does not contain any tissue cross-reactiveM epitopes from the B repeat area of the M protein and thus should not be able to provoke autoimmune-like responses. Other approaches to vaccines are promising but have not yet been studied in humans. A vaccine that contains epitopes of the carboxy-terminal C repeat area of the M protein cross-reacts with most of the common M types but also avoids the tissue-reactive B repeat area.[222] Another vaccine uses a lipid core as a carrier for the C repeat peptide, which has the advantage of not requiring a vaccine adjuvant.[223] A vaccine against the group A carbohydrate is

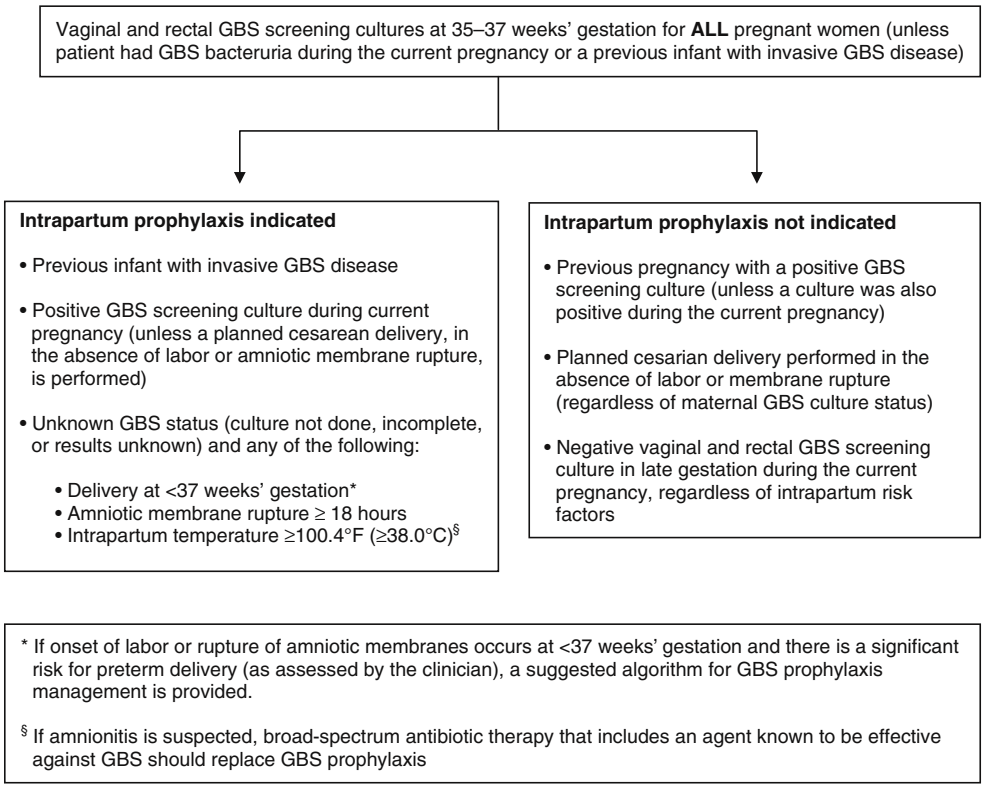

Vaginal and rectal GBS screening cultures at 35–37 weeks' gestation for ALL pregnant women (unless patient had GBS bacteruria during the current pregnancy or a previous infant with invasive GBS disease)
Intrapartum prophylaxis indicated
• Previous infant with invasive GBS disease
• Positive GBS screening culture during current pregnancy (unless a planned cesarean delivery, in the absence of labor or amniotic membrane rupture, is performed)
• Unknown GBS status (culture not done, incomplete, or results unknown) and any of the following:
• Delivery at <37 weeks' gestation*
• Amniotic membrane rupture ≥ 18 hours
• Intrapartum temperature ≥100.4°F (≥38.0°C)§
Intrapartum prophylaxis not indicated
• Previous pregnancy with a positive GBS screening culture (unless a culture was also positive during the current pregnancy)
• Planned cesarian delivery performed in the absence of labor or membrane rupture (regardless of maternal GBS culture status)
• Negative vaginal and rectal GBS screening culture in late gestation during the current pregnancy, regardless of intrapartum risk factors
* If onset of labor or rupture of amniotic membranes occurs at <37 weeks' gestation and there is a significant risk for preterm delivery (as assessed by the clinician), a suggested algorithm for GBS prophylaxis management is provided.
§ If amnionitis is suspected, broad-spectrum antibiotic therapy that includes an agent known to be effective against GBS should replace GBS prophylaxis

Figure 4. Indications for intrapartum antibiotic prophylaxis to prevent perinatal GBS disease under a universal prenatal screening strategy based on combined vaginal and rectal cultures collected at 35- to 37-week gestation from all pregnant women. (From Centers for Disease Control and Prevention.[237])

enhanced by conjugation to tetanus toxoid to produce antibodies presumably protective against all strains.[76] A vaccine against the fibronectin-binding protein (FB54) targets a surface protein strongly associated with virulence.[224] Vaccines against the C5a peptidase of either group A or group B streptococci might not be directly protective but could reduce the effects of the more serious invasive infections.[225]

Group B streptococcal polysaccharide vaccines have been under investigation for over two decades.[226] Although intrapartum antibiotic prophylaxis has been fairly successful, it fails to prevent about 25% of early-onset infections and does not late-onset disease. Vaccines are projected to offer broader more cost-effective protection.[227] The goal of the vaccine approach is to immunize women in order to induce antibody that will later be capable of protecting the fetus against early-onset disease and perhaps afford protection for several months against late-onset disease. Ideally, a vaccine should induce antibodies of the IgG class to cross the placenta and be effective against all of the major serotypes. The first purified polysaccharide vaccine, against type III, was only modestly immunogenic. The immunogenicity was significantly improved by conjugation to tetanus toxoid, as was so successful with *Hemophilus influenzae* and pneumococcal vaccines. Phase I and phase 2 clinical trials have been successfully conducted with individual conjugates of types Ia, Ib, II, III, and V, and preclinical testing has been done with several multivalent vaccines.[226] Several surface proteins of known association with virulence are also being considered. These include several proteins common to nearly all strains, such as alpha, Sip, Rib, and the C5a peptidase, and the c protein, which occurs mainly with type Ia and Ib strains.[44,228] Assuming the eventual development of an appropriate vaccine, it will still be necessary to carefully define the target population. There is yet little enthusiasm for administering vaccines to pregnant women, mainly because of safety and liability issues.[229] It is possible that the target population would have to focus on or include nonpregnant women of childbearing age. The prospects for protecting the more premature fetus is uncertain, since only a minimal amount of maternally derived antibody is able to cross the placenta before the last trimester.[193] Nevertheless, it would be reasonable to suggest that any degree of protection afforded the mother would indirectly affect the fetus.

Pneumococcal vaccines have been studied for nearly 80 years. The 23-valent pneumococcal polysaccharide vaccine licensed in 1983 has been moderately successful in preventing invasive disease adults but has had little effect on nonbacteremic pneumonia.[230] A pediatric 7-valent conjugate vaccine (containing types 1 or 4, 6B, 9V, 14, 18C, 19F, and 23F) was introduced in the United States in 2000, resulting in a decrease of at least 60% in invasive pneumococcal disease among children less than 2 years of age.[170] Wide application of this vaccine has also benefited older adults by producing "herd immunity" and reducing spread.[171] However, nonvaccine serotypes are starting to creep into the population, including a particularly virulent 19A strain.[117] The need for a more universal pneumococcal vaccine is apparent. Several pneumococcal surface proteins, such as PspA, are currently being studied as candidate vaccine for humans.[103,231]

9.8. Prevention of Dental Caries

Conventional approaches to caries prevention have been succinctly reviewed in the Canadian Task on the Periodic Health Examination.[147] Primary prevention includes fluoride, fissure sealants, dietary counseling, oral hygiene, and identification and care of individuals at high risk of developing dental caries. Patients at greatest risk are those with bulimia, Sjögren's syndrome, chemotherapy, radiation therapy, or use of drugs that reduce saliva flow over long periods.

Fluoride-containing toothpastes afford considerable protection against dental caries and are the now most widely used method of providing fluoride. Fluoridation of drinking water at levels of 0.7–1.2 ppm remains an effective public health measure but is not without controversy. About two-thirds of the US population receive fluoridated water, compared to 40% in Canada, 10% in the United Kingdom and in Spain. France does not fluoridate, and Switzerland has replaced fluoridation with fluoride in domestic salt. Fluoride mouthwashes are probably of marginal value in communities with fluoridated water. They are not intended for children under 5 years of age, who may ingest excessive amounts and be in danger of fluorosis. Professional application of topical fluoride may be helpful for high-risk individuals and patients with active caries but is not currently recommended for routine use because of the overall decline in caries incidence.

Sealing of dental pits and fissures with synthetic resins has been done for over 15 years with modest success. Sealants do not add significant protection to initially sound tooth surfaces but may add to further reductions in caries where rates are already quite low.[146] This approach is recommended for selected high-risk patients whose permanent molars have erupted within the previous 2–3 years.[147]

Traditional brushing and flossing has little or no effect on caries prevention, except as a method of applying fluoride in the dentifrice. Professional cleaning (dental prophylaxis) does not prevent caries, although it may be useful for removing stains or calculus. The effectiveness of dietary counseling aimed at reducing intake of sucrose has not been established. Avoiding prolonged use of baby bottles and bedtime bottle propping may prevent "nursing caries." Nevertheless, it is now recognized that parental attitudes and beliefs play a key role in moderating oral health-related behavior in young children.[148]

Vaccines against dental caries, based on antigens derived from *S. mutans,* have been under investigation for a number of years.[184,232] Experimental vaccines have been successful in preventing caries in animals. Candidate vaccines include surface adhesins (Ag I/II, antigen B), glycosytransferases (GTF) that synthesize adhesive glucan from sucrose, and glucan-binding proteins. Several small clinical trials have demonstrated the feasibility of similar vaccines in humans. GTF vaccines given orally, intranasally, or topically applied to tonsils have been shown to induce salivary IgA antibody responses in humans, but long-term evaluations of efficacy have not been done. The major reason is that dental caries is not a life-threatening disease, and potential benefits must clearly outweigh even theoretical concerns of safety.

10. Unresolved Problems

Despite the availability of antibiotics, the diagnosis and treatment of group A streptococcal pharyngitis continue to pose problems for the clinician. The distinction between infection and carrier state is frequently blurred. The new rapid diagnostic tests make it possible to treat promptly and reduce clinical symptoms, but it is unlikely that changes in clinical practice have influenced the recent resurgence of acute rheumatic fever or of severe invasive streptococcal disease and toxic shock. Further study of epidemiologic factors, pathogenic mechanisms, and host response is clearly needed. Poststreptococcal reactive arthritis and its less certain relationship with cardiac sequelae continues to present a clinical dilemma for clinicians.[208] Group A streptococci, along with staphylococci, have been suggested as an etiology for Kawasaki disease, a vasculitis syndrome that occurs mainly in children. It is hypothesized that staphylococcal toxin TSST-1 or streptococcal exotoxins act as superantigens and cause aberrant amplification of certain T-cell lines implicated in the disease process.[233]

Group B streptococci remain the most common cause of serious bacterial disease in newborn infants in many western countries. Despite similar maternal colonization rates and

presumably similar risk of exposure, the disease is infrequent in some areas of Europe and Asia. The reasons for this are not clear but could be of considerable practical importance. Methods for intrapartum antibiotic prophylaxis have been established but remain to be implemented on a wide scale. Meanwhile, we still have little understanding of the local and systemic defense mechanisms required to prevent infection by this usually benign organism. Other streptococci, particularly oral and enteric species, are constant companions that cause disease only under specialized circumstances. It is not clear whether some of these organisms are in fact "virulent" in certain settings, or whether the disease is due mainly to predisposing conditions in the host. The question may be moot, but answers would help us direct attention to better ways of preventing nosocomial and opportunistic infections.

The unanswered questions apparent from the discussions in this chapter remain: how do streptococci cause disease, and why do some individuals become infected while others do not? Many pathogenic strains of group A *Streptococcus* have been sequenced and genes corresponding to virulence factors have been catalogued. However, the regulation of these genes has not been adequately investigated. In addition, major virulence factors, such as exotoxins, enzymes, M proteins and polysaccharide capsules, have been identified, but the pathophysiologic mechanisms by which they interact with the host remain uncertain and do not completely explain the disease processes. The determinants of colonization, the defenses of the host at the mucosal level, and the development of local and systemic immunity are not well understood. More needs to be learned to facilitate the development of effective vaccines and implement other preventive measures.

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CHAPTER 36

Syphilis

Anne Rompalo and Willard Cates

1. Introduction

Few fields of medicine have been more carefully scrutinized by experts than syphilis.[1–4] Whether in economic, political, social, ethical, or epidemiologic arenas, syphilis has been the prototypic sexually transmitted disease (STD) control program of the 20th century.[4] Thus, the approaches taken and the measures used have been the model for control programs directed toward other STDs. In addition, many other clinical preventive services, such as those for tuberculosis, immunization, maternal and child health, and human immunodeficiency virus (HIV), have used concepts derived from syphilis control activities in their own prevention strategies. However, much of the epidemiological knowledge about syphilis control occurred prior to the 1950s.[4]

This chapter reviews the factors affecting syphilis epidemiology and control, including historical tradition, epidemiological bases, screening approaches, clinical management, and potential for immunization. It concludes with a consideration of future directions.

2. Historical Aspects

2.1. Early History

Syphilis carries a controversial history, not only because of its prurient nature but also due to its alleged origins. Two theories predominate. The unitarian theory holds that syphilis, like yaws, began in equatorial environments, being transmitted by casual contact. However, when the organism migrated to colder climates where more clothing was worn, sexual transmission provided the main avenue for contact with *Treponema pallidum*. The Columbian theory holds that syphilis arose in North America and was brought back to Europe around the beginning of the 16th century. It later received its current name from a poem written in 1530 about an infected shepherd called Syphilis.

History is replete with the names of illustrious individuals who may have contracted syphilis, but folklore implies a tenuous veracity.[5] Documentation of the symptoms and signs of the illnesses is usually poor, and specific diseases are not identified as such. Nonetheless, syphilis, like smallpox, produced measurable historical consequences. The fates of national armies, royal succession, and international explorations have all been affected.

The key discoveries involving syphilis occurred in Germany in the early 20th century. Within 10 years, the spirochete was identified (1905), the Wasserman reaction developed (1906), and Salvarsan discovered (1909). Knowledge in this field progressed rapidly with acceptance of the germ theory, development of serological tests, and the use of toxic cures. At the same time, the increasing professionalization of medicine coincided with the rise of venereology as an established specialty. Syphilis became the disease on which the skills of 20th-century physicians were judged.

2.2. Social Attitudes

Throughout history, syphilis has caused society to wrestle with moral issues associated with public health problems. Fortunately, in recent years, the human tragedies suffered by those with syphilis have led to more effective social and political responses designed to interrupt transmission and reduce complications, rather than to stigmatize those suffering from disease.[4]

During the past century, American society evolved toward a more tolerant view of those with syphilis.[4] Before

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1900, physicians were known to withhold treatment for the illness, fatalistically regarding infection as evidence of (and even punishment for) promiscuous behavior. During the early 1900s, the "social hygiene" movement highlighted syphilis as a major public health problem; although illicit sexual forays were still scorned, at least the consequences of sexual intercourse could be both discussed publicly and treated professionally. Campaigns were waged against prostitution, and condoms were marketed as prophylaxis against infection (and pregnancy).

In the New Deal era, Surgeon General Thomas Parran seized an opportunity to dramatize the plight of those with syphilis.[1] With the availability of penicillin and the higher moral imperative of winning a world war, during the 1940s, the image of syphilis shed even more of its stigmatic cloak. Syphilis could be diagnosed and treated on an outpatient basis, thus allowing a more positive attitude toward finding and treating both patients and partners alike. During the most recent decades, the changing role of women and homosexuals in society was accompanied by a shift in the nation's attitude toward human sexuality. This created a growing constituency of those demanding personal and public health solutions, as well as an increasing sympathy for the "innocent victims" of the late sequelae. Thus, at the present time, social concerns about syphilis have a broad community base.[6]

2.3. Control Programs

In the United States, the first national syphilis control efforts began in 1918 when Congress passed the Chamberlain–Kahn Act, creating the Venereal Disease Division of the US Public Health Service.[4] Passage of this act was stimulated by World War I, when syphilis became recognized as a major cause for rejection from the armed services. However, the end of the war brought a decline of interest in the disease, and an effective national program never really began. Twenty years later, Parran's influence resulted in the passage of the National Venereal Disease Control Act, which provided annual grants-in-aid to individual states. This law established the basic model for federal, state, and local cooperation for venereal disease control efforts that remain in use today.[6]

During the 1940s, the primary objective of syphilis control programs was to prevent the late complications of the disease.[6] Public awareness programs set the stage for mass serological testing programs to detect infected individuals, who were then generally treated with heavy metals. Beginning in 1943, the so-called "Rapid Treatment Centers" were established by the federal government.[4] These inpatient facilities provided a course of 5- to 10-day arsenical therapy instead of the 70 weeks required on an outpatient basis. Soon thereafter, however, the availability of penicillin for widespread use contributed to making the syphilis control program even more successful. By the early 1950s, the reservoir of untreated or inadequately treated syphilis had been dramatically reduced. In succeeding years, the incidence of complications declined markedly. Over the next two decades, deaths attributable to syphilis decreased by 85%, and admissions to hospitals for syphilitic psychoses declined by 97%.[3] Congenital syphilis, for which somewhat better data are available, declined by 90% between 1941 and 1972.[7]

As the prevention of late complications became a reality, the interruption of syphilis transmission in its infectious stages emerged as the primary objective of control programs. The focus for public health officials shifted from lowering syphilis prevalence to reducing its incidence.[3] The strategy for accomplishing this has gradually evolved since the late 1950s. Building on the concept of case finding through routine blood testing, syphilis control programs increasingly emphasized case prevention by aggressive treatment of sex partners. Patients in the infectious stages of syphilis were interviewed to identify exposed sex partners, who were then offered treatment (termed "epidemiological treatment") before they could either develop symptoms and/or spread the infection. While this approach was successful in stemming outbreaks and reducing community levels of syphilis, it was costly in financial and human resources. Thus, syphilis control (like other public health programs) was vulnerable to the flow of financial support.

2.4. Funding Levels and Syphilis Incidence

State and local resources mainly support laboratory, diagnostic, and treatment services for syphilis. After adjusting for inflation, these nonfederal resources have remained relatively stable during the past 40 years. However, federal appropriations for STD control (not including HIV), which support the surveillance and outreach activities, have been more variable. From the high of over $17 million (absolute dollars) in 1949, resources decreased quickly to $13 million 1 year later, to $700,000 in 1955, when the availability of penicillin permitted the closing of the Rapid Treatment Centers and when sharp declines in early syphilis incidence suggested that the problem had been "solved."[3]

The optimism was ill founded; infectious syphilis increased in the late 1950s. A Surgeon General's Task Force considered the problem in 1961 and recommended a 10-year eradication program.[2] Federal grants to states for syphilis

control were increased from $2.4 million in 1960 to $6.3 million in 1965, and remained at this level until 1970. This effort was associated with a decline in syphilis incidence between 1965 and 1969. However, inflation led to sizable reductions in purchasing power, and infectious syphilis rates began to increase again in the 1970s.[7]

After 1970, resources for syphilis control were further eroded by the expanding spectrum of infections recognized as being sexually transmitted.[8] Although additional funds were appropriated for gonorrhea control in the 1970s and for chlamydia control in the 1980s, these new resources were never enough to support all the necessary STD control activities. Policymakers were forced to choose alternatives among secondary prevention approaches for the different organisms. Because of the greater burden of disease caused by gonorrhea, chlamydia, and viral STDs, syphilis was relegated to receive a smaller share of the STD resources during the 1970s and 1980s. In recent years, syphilis has received both an absolute and a proportional increase in federal STD funds.[8] In 1998, after a steady decline in incident syphilis rates to historic lows, a national plan to eliminate syphilis from the United States was initiated.

2.5. The National Plan to Eliminate Syphilis from the United States

The goal of the syphilis elimination effort at the national level was to reduce primary and secondary syphilis cases to 1,000 or fewer and to increase the number of syphilis-free counties to 90% by 2005. Five strategies were considered critical: enhanced surveillance, strengthened community involvement and partnerships, rapid outbreak response, expanded clinical and laboratory services, and enhanced health promotion. Between 1999 and 2004, CDC invested in excess of $107 million to high syphilis morbidity areas in the United States to support infrastructure, interventions, evaluation, research, community outreach, and clinical and laboratory services.[8]

Since the plan has been issued, declines in syphilis rates among key populations at risk have occurred. Between 1999 and 2004 primary and secondary syphilis rates decreased by 37% among blacks; the black–white racial disparity in syphilis rates fell from 28.6:1 to 5.6:1; rates among women overall fell 60%, from 2.0 to 0.8 cases per 100,000; congenital syphilis rates declined 39%, from 14.5 to 8.8 per 100,000 live births.[8]

Although the syphilis elimination effort has been extremely successful in decreasing syphilis among heterosexuals, since 1999 rates have been increasing among men who have sex with men (MSM). Sexual networks of MSM who are infected with syphilis are marked by high volume of sexual contact between the core and periphery. Infected MSM migrate across the United States, thus facilitating the introduction of infection into new susceptible subpopulations. The ability of MSM to identify sex partners over the Internet further complicates control strategies since the Internet facilitates anonymous sex and reduces the time required to form sexual links.[9]

The evolving epidemiology of syphilis in the United States, changing risk groups, and social environments present challenges for syphilis elimination program activities.[8] Syphilis is now diagnosed more frequently in the private as opposed to public sector, which raises concerns regarding rapid identification, reporting, and counseling as well as partner management. Public sector health services are currently facing increasing demands with decreasing financial resources. In response to this changing epidemic, the CDC has revised its broad syphilis elimination efforts and plans to target interventions to populations at greatest risk, and approve accountability of prevention efforts.[8]

3. Methodology

3.1. Sources of Mortality and Morbidity Data

Syphilis mortality estimates are derived from death certificates, as reported by the National Center for Health Statistics (NCHS). The accuracy of these data over time is influenced by changes in coding methods, diagnostic approaches, physician reporting candor, and autopsy percentages. Thus, the validity of mortality case definitions may vary over time, place, and patient population.

Syphilis morbidity data are collected by the Centers for Disease Control and Prevention (CDC) through formal surveillance systems based in the states. Data may vary in accuracy, depending on the priorities of STD control programs. These morbidity data tend to be more accurate in states that have laws that require reporting of positive syphilis serological tests.[3] Because private physicians are less reliable sources of morbidity data, the number of reported syphilis cases overrepresents patients seen in the public sector, and thus underestimates disease in the more affluent and older patient populations. Since public health partner notification activities are directed at interrupting infectious syphilis, morbidity of early syphilis is more accurate than late or latent disease.

Data on private sector case rates and disease sequelae can be derived from three annual sources: (1) the Hospital Discharge Survey of NCHS, which includes 7500 randomly selected hospitals from throughout the United States; (2) the

National Ambulatory Medical Care Survey of NCHS, which is a probability sampling of the diagnoses of 1900 physicians; and (3) the National Disease and Therapeutic Index (NDTI), which is a private survey of a random sample of office visits to US physicians in office-based practices. The major shortcoming of these databases is relatively small numbers of syphilis cases, which leads to wide confidence intervals in subpopulations.

3.2. Serological Surveys

Our syphilis seroepidemiological tools are inexpensive, relatively simple to perform, highly sensitive and specific, and readily available. Positive screening tests are generally followed by a variety of confirmatory tests to substantiate the diagnosis. Widespread serological testing is usually a poor case-finding tool for early infectious syphilis, but may be a good method for disease surveillance.

3.3. Laboratory Diagnosis

3.3.1. Identification of the Organism. In vitro culture of *T. pallidum* is not currently possible for diagnostic purposes. However, darkfield microscopic identification of the motile *T. pallidum* in secretions from primary, secondary, and early congenital lesions constitutes a rapid, reliable, and specific test. *Treponema pallidum* can also be identified from lesions and in biopsy material by commercially available direct fluorescent antibody (DFA) techniques. DFAs advantage is that the specimen can be transported to a laboratory where an experienced microscopist can make the proper identification. This procedure is ideally suited for testing oral and anorectal lesion secretions because it will differentiate *T. pallidum* from *T. denticola* and other spirochete strains.

False-negative darkfield and direct fluorescent antibody tests may occur even with adequate clinical specimens. Self-treatment with penicillin, for instance, can render a chancre darkfield-negative within hours. The same occurs when antiseptic ointments are locally applied to syphilis lesions.

3.3.2. Serology. Serological tests are valuable in syphilis diagnosis when ulcerative lesions are not available for darkfield examination. In primary syphilis, all serological tests are less sensitive than direct visualization of *T. pallidum*. When syphilis has reached the secondary stage, antibody levels have risen and nearly all serological tests are reactive.

Based on the character of the antigen, two major categories of syphilis serological tests exist. The first syphilis antigen is a defined combination of cardiolipin, cholesterol, and lecithin. These nontreponemal tests have similar sensitivities and specificities and include the Venereal Disease Research Laboratory (VDRL) and the rapid plasma reagin (RPR) tests.

The second category of syphilis serologies comprises the treponemal tests. Intact *T. pallidum* organisms are the antigens used in the *T. pallidum* immobilization (TPI) and fluorescent treponemal antibody absorption (FTA-ABS) tests. The TPI test is labor-intensive and is now used only in research laboratories. The *Treponema pallidum* particle agglutination assay (TPPA) is one of the newer confirmatory tests on the market. Recently the feasibility of using oral fluid as a specimen for TPPA testing was evaluated, but an approximate eightfold loss of assay sensitivity was noted when using oral fluid compared to serum for TPPA.[10]

False-positive nontreponemal tests are common and are usually of low titer ($\leq$1:4). False-positive nontreponemal tests are divided into acute and chronic, based on whether reactions revert to negative in less than 6 months. False-positive treponemal tests can also occur, usually associated with lupus erythematosus, pregnancy, and intravenous drug use.

4. Biological Characteristics of the Organism

Treponema pallidum is classified in the order Spirochaetales, genus *Treponema*. The latter includes both human pathogens and nonpathogenic organisms. The pathogens and the diseases they cause are as follows: *T. pallidum* (syphilis), *T. pertenue* (yaws), *T. carateum* (pinta), and *T. pallidum* variant (endemic syphilis). Morphologically, these four treponemes are identical. DNA homology has demonstrated a close similarity between *T. pallidum* and the other pathogens, but marked differences between the pathogens and the nonpathogenic treponemes.[11]

Cross-resistance between *T. pallidum* infection and the other treponemal infections suggests immunologic similarities. Syphilis nontreponemal and treponemal tests are reactive in patients with yaws, pinta, and endemic syphilis. Syphilis incidence is low in areas where yaws is endemic, but has increased after active yaws eradication program. The pathogenic treponemes can be differentiated by the host response in various animal models.

Treponema pallidum, like other treponemes, is a gram-negative bacterium. Its length varies from 6 to 15 μm, possibly because its reproductive cycle consists of growth in length followed by division half-way along its long axis. *Treponema pallidum* is a fragile organism, which is readily destroyed by disinfectants commonly used in laboratories and clinics. *Treponema pallidum* utilizes oxygen

and is microaerophilic. None of the human pathogenic treponemes have been grown in broth or on bacteriological media.

In 1998 the genome of *T. pallidum* was sequenced.[12] Its genome is relatively small suggesting that it utilizes hot biosynthesis to fulfill some of its metabolic needs.

While biological functions are suggested for only about 55% of *T. pallidum* 's 1041 open reading frames, even these relatively early studies offer important insights into syphilis pathogenesis. A family of repeat genes, the *Tp* genes, encode proteins homologous to the major sheath proteins of *T. denticola.* Antibodies to the *TprK* variable regions are protective in a rabbit model. With successive passage, increasing diversity is observed in the *TprK* V region genes. Antigenic variation through gene conversion has been hypothesized to be one mechanism of escaping immune surveillance, allowing for prolonged infection and persistence in the presence of a robust host response. Human and animal studies suggest that a Th1 response is elicited in primary syphilis. Progression to the secondary stage is accompanied by a shift to a Th2 response, allowing for incomplete clearance of the pathogen. In pregnancy, intense inflammatory responses and prostaglandins induced by fetal infection may be responsible for fetal death or pre-term delivery and severe growth retardation or other manifestations of congenital syphilis. Understanding of the molecular targets of these immune responses may facilitate the development of vaccines for syphilis.[13]

5. Descriptive Epidemiology

Because syphilis passes through both clinical and subclinical phases, case definition presents a dilemma for surveillance purposes. Trends in primary and secondary (P&S) syphilis provide a measure of disease incidence, since their symptomatic clinical presentations are of relatively short duration. This is especially true in men, where the chancre most often occurs on the external genitalia. In contrast, primary infections of women are reported less commonly. Latent disease rates, on the other hand, depend on the levels of screening and partner notification activities. Thus, trends in latent disease rates may reflect the levels of resources devoted to syphilis control activities.

5.1. Prevalence and Incidence

After the advent and widespread use of penicillin treatment, syphilis cases dropped dramatically.[7] Fifteen years after the introduction of penicillin, the number of syphilis cases had dropped by 90% (Figure 1). Cases reached their nadir in 1955. Thereafter, syphilis ebbed and flowed during the next 30 years as rates increased, then decreased in the gay male population. In the second half of the 1980s, heterosexual syphilis transmission was fueled by the crack-cocaine epidemic, and rates reached their highest levels since post-World War II. However, since 1991, P&S syphilis declined markedly; in 1994, just over 20,000 cases were reported, the lowest number since 1977.

Since 1999, substantial reductions in rates in women and newborn infants have occurred. Rates of P&S syphilis declined from 2.0 to 0.8 per 100,000 women. Rates in African-Americans fell from 14.3 to 8.9 per 100,000 population and the Black:White racial disparity was reduced from 28.6:1 to 5.6:1 (Figure 6). However, recent resurgence of syphilis among MSM has occurred since 1999 and in 2004, more than 60% of new diagnoses of P&S syphilis were estimated to occur in MSM, with HIV-positive MSM bearing a disproportionate burden of disease. Between 2004 and 2005, the national P&S syphilis rate increased by 11.1%, from 2.7 to 3.0 cases per 100,000 population, and the number of reported cases increased from 7,980 to 8,724. This increase has been reported for most age groups, for both sexes but more so among men, and for the second consecutive year the P&S rate has increased among blacks, with the largest increases among black men (from 13.9 per 100,000 in 2004 to 15.7 per 100,000 in 2005).

Congenital syphilis cases have paralleled the trends in female P&S disease (Figure 2). After 1988, the number of congenital syphilis cases increased dramatically, not only because of the rising incidence in women but also because of a broader case definition begun that year. However, because of the declining number of adult syphilis cases in the first half of the 1990s, congenital syphilis cases fell as well.[7,8] Between 1991 and 2004 congenital cases declined by 92% (107.3 cases per 100,000 live births to 8.8 cases per 100,000 live births).[8] Overall rates of congenital syphilis decreased by 12.1% between 2004 and 2005, from 9.1 to 8.0 per 100,000 live births.

5.2. Contagiousness

Except for intrauterine infection, syphilis is transmitted by sexual contact between infected and uninfected epithelial surfaces. The transmission rate is relatively high, especially after repeated exposures, and is inversely proportional to the stage of the disease. Among couples with frequent sexual contact, four out of five partners exposed to the infectious lesions of early syphilis were infected; less frequent transmission occurred when the index case had been infected for more than 4 years.[14,15] Among untreated nonconjugal sex

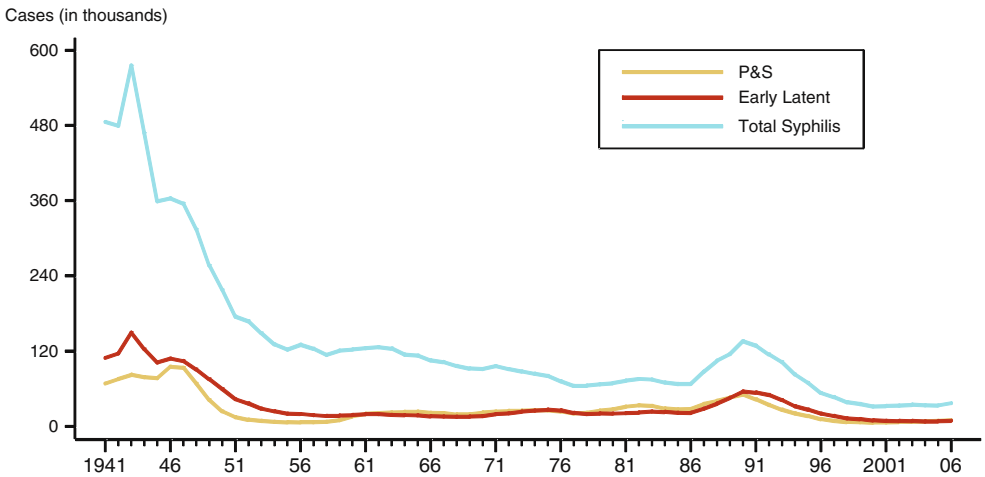


Figure 1. Syphilis – reported cases by stage of illness: United States, 1941–2006.

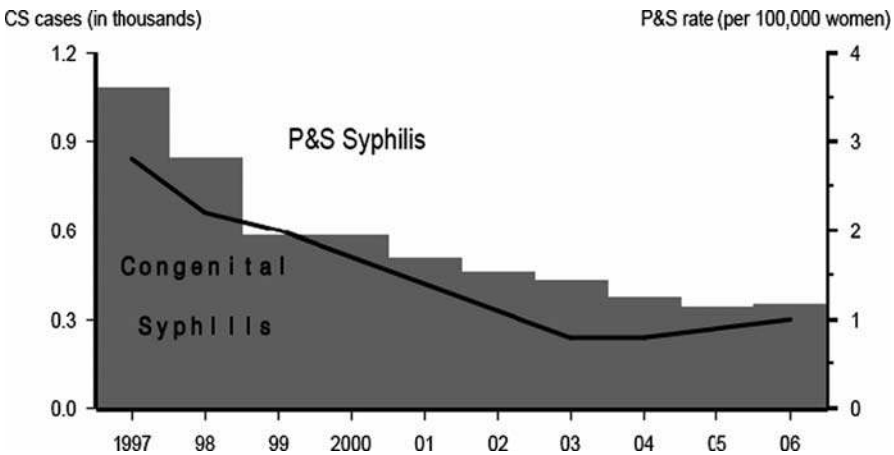


Figure 2. Congenital syphilis – reported cases in infants <1 year of age and rates of primary and secondary syphilis among women: United States, 1997–2006.

partners having less frequent exposure to patients with P&S syphilis, 30–40% acquired infection.[6,16]

The infectious dose for the transmission of *T. pallidum* infection is relatively low. The ID_{50} of the rabbit-acclimated Nichols strain for the production of intracutaneous infection in volunteers is about 50 organisms.[17]

Perinatal transmission of syphilis occurs as early as the ninth week of gestation.[14] The risk of intrauterine transmission of infection in maternal syphilis is high, and it varies with the stage of maternal disease. The risk of congenital syphilis acquisition is 50% in maternal P&S infections, 40% in early latent infections, and only 10% in late latent infection.[18] Healthy term infants were born to 10% of the early latent maternal infections and to 70% of those with late latent disease.

5.3. Geographic Distribution

Currently, the southern United States continues to have a higher rate of P&S syphilis than any other region. Cases in the South account nearly half of total P&S syphilis cases reported (Figure 3). Of 706 counties reporting at least one case of P&S syphilis in 2005, 5 (0.7%) had rates at or below the Healthy People 2010 target of 0.2 cases per 100,000 population. The 701 counties with rates above the Health People 2010 target accounted for 99.9% of the total P&S syphilis cases reported in 2005 (Figure 4).

5.4. Temporal Distribution

A seasonal trend pattern has not been demonstrated for syphilis.

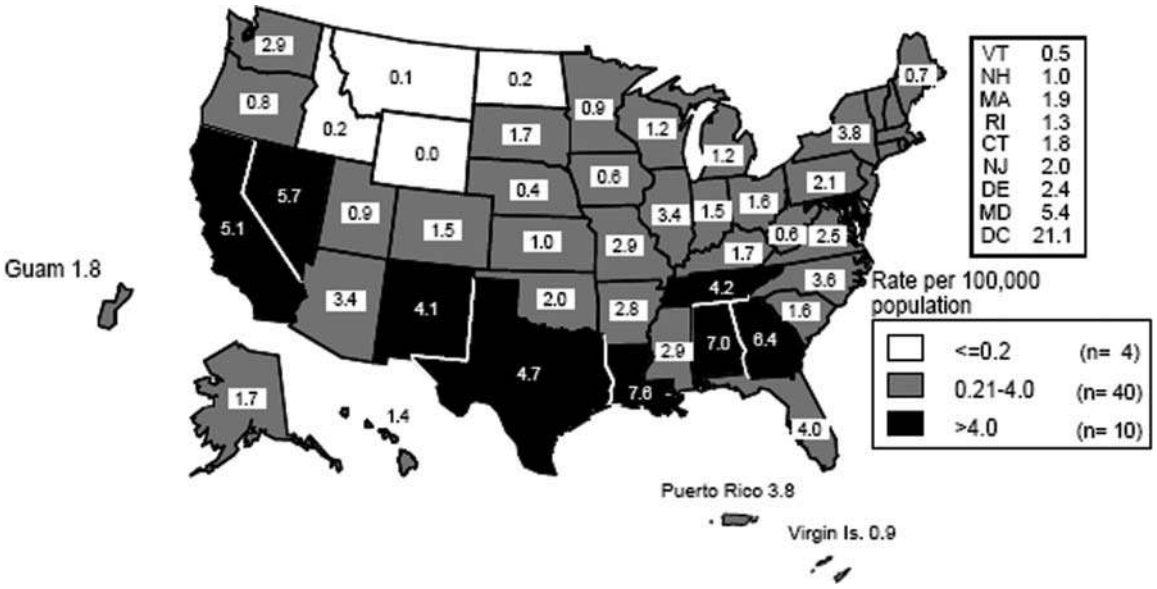


Figure 3. Primary and secondary syphilis – rates by State: United States, 2006.

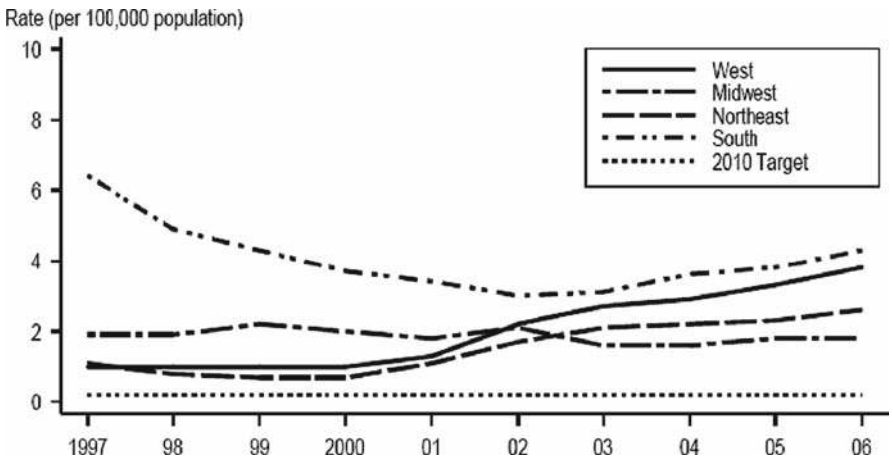


Figure 4. Primary and secondary syphilis – rates by Region: United States, 1997–2006 and the year 2010 objective.

5.5. Age and Gender

In 2005, the rate of P&S syphilis was highest among women in the 20- to 24-year-old age group (3.0 cases per 100,000 population) and among men in the 35- to 39-year-old age group (13.2 cases per 100,000 population) (Figure 5).

5.6. Race

Nearly all studies have found markedly higher syphilis case rates in African-Americans compared to whites. Recent trends showed African-American syphilis rates increasing markedly between 1985 and 1990, but declining precipitously thereafter, with a marked reduction in the Black:White ratio after the National Syphilis Elimination efforts began in 1999 (Figure 6). The reasons for the higher syphilis rates in African-Americans are currently not known. Racial and economic segregation help define sociosexual networks with an elevated prevalence of disease, thus maintaining high levels of infection.[19]

5.7. Occupation

With the exception of commercial sex workers, occupation bears no specific relationship to the risk of acquiring

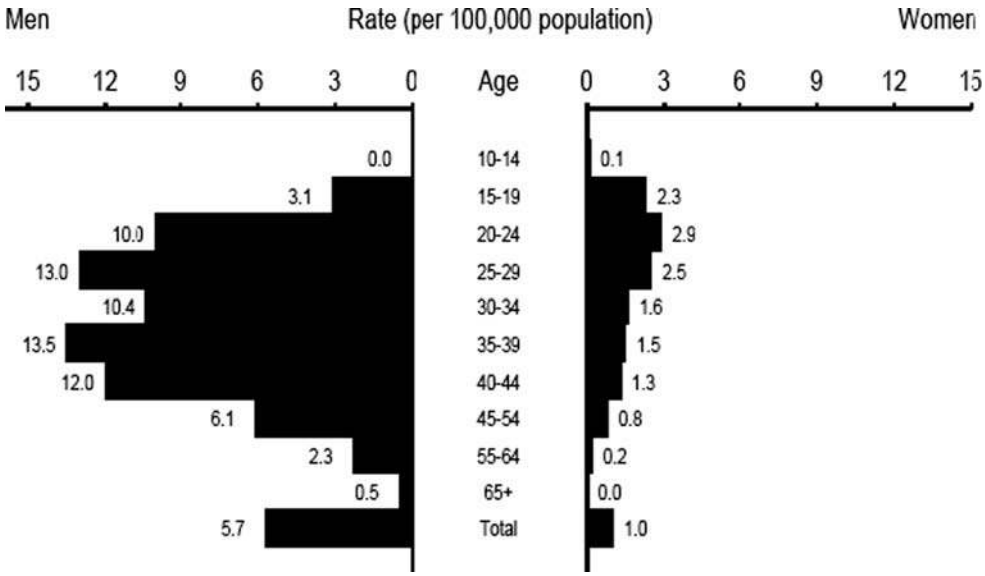


Figure 5. Primary and secondary syphilis – age- and gender-specific rates: United Stated, 2006.

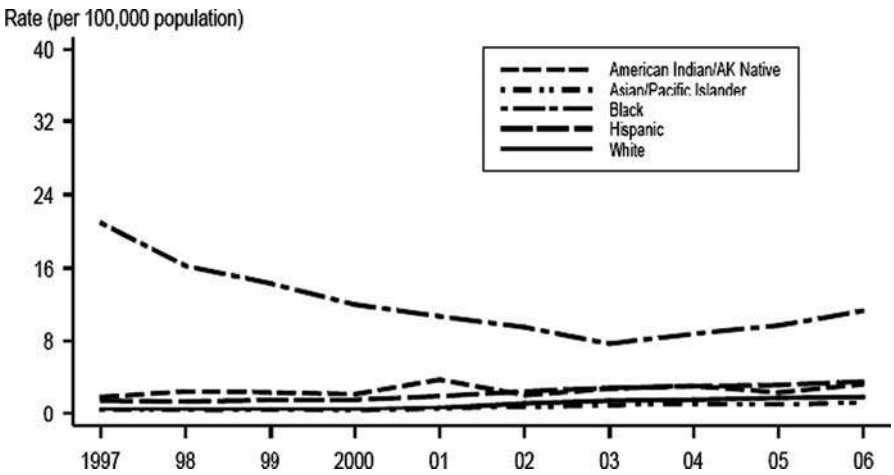


Figure 6. Primary and secondary syphilis – rates by race and ethnicity: United States, 1997–2006 and the year 2010 objective.

syphilis. Occupations that employ younger, sexually active individuals, especially from syphilis endemic areas, would likely yield higher infection rates.

5.8. Occurrence in High-Risk Environments

Over the past several decades, syphilis outbreaks have occurred in settings associated with high numbers of anonymous sex partners: the bathhouses serving gay males and crack houses serving low-income populations where sex is exchanged for drugs.

5.9. Socioeconomic Factors

While all social strata are at risk for sexually transmitted infections, syphilis has become entrenched in the poor.[19] Factors such as educational and income levels, which are directly related to the frequency of using prophylactic and therapeutic measures to prevent disease, are often important

determinants of the prevalence of syphilis and its transmission dynamics. Moreover, health-care seeking is also directly related to income level.

6. Mechanisms and Routes of Transmission

Treponema pallidum is transmitted during sexual contact between an infectious individual and a susceptible recipient. Infection of a sex partner occurs in about one-third of coital exposures. Initial infection fails to occur unless adequate numbers of treponemes are transferred to the partner's mucosa or abraded skin. No natural resistance was found in experimental infection of volunteers not previously exposed to *T. pallidum*.[17]

Infected individuals are not continually infectious to others. Patients may remain infected with *T. pallidum* from the time of inoculation until their death, but they are infectious to others primarily during the several weeks of the P&S stages of syphilis; late latent and late syphilis are not infectious stages.

For syphilis infection to be transmitted, adequate numbers of *T. pallidum* must be on the surface of the organ that contacts the recipient's skin or mucosae. The exact number needed to infect is unknown, but experimental intracutaneous injection of the agent required only 57 organisms.[17] *Treponema pallidum* can penetrate mucosal surfaces, but penetration is resisted by intact skin. It can penetrate through the skin abrasions that occur in sexual activity. Rabbits resist syphilis when *T. pallidum* is placed on normal skin, but the minimal trauma associated with shaving the animal allows the same skin to be infected.

In childbirth, a similar situation may occur when *T. pallidum* from a mother's genital chancre is transmitted to the child as it passes through the vagina. Under this circumstance, the chancre(s) in the newborn develops several weeks postpartum in the site(s) of birth trauma, such as where extraction forceps were applied to the head. The more common mode of congenital syphilis transmission is via a maternal septicemia with passage of *T. pallidum* through the placenta and into the developing fetus. Such a septicemia is most likely when the pregnant mother has primary or secondary syphilis. Treatment of an infectious mother in the first trimester may prevent congenital syphilis, because placental passage usually occurs during the second and third trimesters.

Hematogenous spread of *T. pallidum* has occurred with blood transfusions, but is quite rare. Treponemes are present in blood for only the short duration of the P&S phases. In addition, blood is screened with a syphilis serological test before it is transfused into a recipient. Fomite transmission of syphilis is virtually nonexistent, because *T. pallidum* is rapidly killed by drying. It has not been found in urine, milk, or sweat.

7. Pathogenesis and Immunity

Treponema pallidum infection evokes both humoral and cellular immune responses. The former's detection and quantification is important in the laboratory diagnosis of syphilis. Treponemal (anti-*T. pallidum*) and nontreponemal (anticardiolipin) antibodies begin developing in primary syphilis, and in the secondary stage all patients have high titers of both antibodies. Titers of anticardiolipin antibodies then start to slowly decline and spontaneously revert to negative in about one-third of untreated patients. Anti-*T. pallidum* antibodies remain detectable for the life of most patients. Cell-mediated immunity also develops in primary syphilis, appears to be suppressed during the secondary stage, and is again detected in the latent and late stages. The TPR K protein of the Tpr family of polymorphic multi-copy repeat proteins has been shown to be a target for opsonic antibody.[20]

The roles of antibodies and cellular immunity in resistance to infection are not completely understood. Human inoculation experiments have shown that syphilis infections confer some degree of resistance to reinfection.[17] Patients treated previously for primary or secondary syphilis were easily reinfected with *T. pallidum* despite having an anamnestic antibody response to both cardiolipin and *T. pallidum* antigens. Patients with untreated late latent syphilis were most resistant; many patients with this stage of syphilis resisted an intracutaneous challenge of 100,000 *T. pallidum*. This compares with an infectious dose of about 50 organisms for an initial syphilis infection. Resistance to reinfection correlated better with cellular immunity than with humoral antibodies.

Resistance to reinfection can be differentiated from chancre immunity. The latter is failure of *T. pallidum* to produce a second chancre while the primary chancre is still present. Chancre immunity may be secondary to cellular immunosuppression in early syphilis.

8. Patterns of Host Response

8.1. Clinical Features

The interval between infection with *T. pallidum* and development of the first clinical sign of syphilis varies from 10 to 90 days, with the typical case developing an ulcer (the primary chancre) in 3 weeks.[21] If someone exposed to

syphilis does not develop either a chancre or a positive cardiolipin serology within 90 days, he/she probably was not infected.

While clinical staging is imprecise, it provides a useful guide to treatment decisions and syphilis control activities. Considerable overlap between stages is common.[21]

8.1.1. Primary Stage. When infection does occur, the first sign of syphilis develops at the site of inoculation and consists of a pink to red macule. This flat lesion rapidly becomes a palpable nodule and within several days its surface erodes to produce the characteristic chancre. Chancres are usually single, without pain and are accompanied by painless regional lymphadenopathy. The asymptomatic character of such a large genital ulcer contrasts markedly with the pain of smaller genital herpes and chancroid ulcers.

A primary syphilis chancre heals spontaneously without treatment, usually within 2–3 weeks, but occasionally may persist for up to 8 weeks. A residual scar in the area of the chancre is rare unless the lesion was secondarily infected. The regional adenopathy requires a longer time to resolve spontaneously.

8.1.2. Secondary Stage. Several weeks after the chancre has healed, the manifestations of secondary syphilis emerge. Nonspecific symptoms of this stage include low-grade fever, headache, sore throat, malaise, anorexia, and arthralgias.[21] The most characteristic secondary syphilis signs, although not pathognomonic, are changes in the skin, hair, and mucous membranes.

Skin lesions are discrete and may be macular (flat) or papular (small palpable elevations), usually on the trunk, the palms, and the soles. Hair loss may occur in secondary syphilis. Patches of scalp hair are thinned rather than completely denuded and the result is a moth-eaten appearance to the scalp. In perineal sites, secondary syphilis lesions are generally nodular and are referred to as condylomata lata, similar to genital warts (condylomata acuminata).

A patient with secondary syphilis may have one, several, or all of the signs of the secondary stage. Since each of the signs may be associated with other diseases, none are specific to syphilis. However, when more of these signs are present, the diagnosis of syphilis is more likely to be accurate. The diagnosis of secondary syphilis is confirmed by the identification of *T. pallidum* in mucosal lesions or by the conversion of a syphilis serological test from negative to positive. Essentially all treponemal and nontreponemal syphilis serologies are reactive in this stage of syphilis.

The manifestations of secondary syphilis last 2–8 weeks and then, as with primary syphilis, heal spontaneously without residual scarring. When healing is complete, the patient enters into a latent phase with no clinical symptoms or signs of syphilis. The syphilis serological tests, however, remain positive.

8.1.3. Clinical (Infectious) Secondary Relapse. About 25% of untreated patients relapse from latency back into secondary syphilis.[22] This usually occurs during the first 5 years of latency, although most relapses take place in the first year. Such relapses are infrequent, possibly related to inadvertent syphilis treatment by systemic antibiotics used to treat another infection. The diagnosis of latent syphilis requires a spinal tap to differentiate it from asymptomatic neurosyphilis. The differential is important because neurosyphilis requires more prolonged therapy, as well as posttherapy spinal taps, to document therapeutic success.

8.1.4. Late Benign Syphilis. Late or tertiary syphilis occurs much less frequently today than was reported in the Oslo study of untreated syphilis.[8,22] As with the reduction in secondary syphilis relapses, this is probably related to inadvertent syphilis treatment when systemic antibiotics are used to treat a variety of other infectious diseases. This trend, however, may be reversing, since concurrent HIV infection may facilitate syphilis progression to symptomatic neurosyphilis.[23] This occurred in both untreated and treated syphilis patients.

One-third of untreated syphilis patients in the Oslo study progressed from the latent stage to one or more of the tertiary stages of syphilis.[22] The remaining two-thirds of syphilis patients remained asymptomatic during the rest of their lives. The sera of some of these asymptomatic patients also become nonreactive in the cardiolipin syphilis serological tests.

Three types of tertiary syphilis occur: gummas, cardiovascular syphilis, and central nervous system syphilis. Gummatous lesions developed in about 15% of the Oslo study patients.[22] These lesions have their onset 2–40 years after primary syphilis. Seventy percent of gummas involve the skin, 10% are found in bone, and a similar percentage are present on mucosal surfaces. Gummas can involve any organ. Skin and bone gummas are disfiguring, but involvement of a vital organ such as the heart or liver could be fatal.

8.1.5. Cardiovascular Syphilis. Cardiovascular tertiary syphilis develops 10–30 years after primary syphilis and was clinically evident in about 10% of the Oslo study's untreated syphilis patients.[22] Another 20–30% of untreated patients have cardiovascular changes that do not produce signs or symptoms. The microscopic pathology of cardiovascular syphilis is similar to that of neurosyphilis: blood vessel inflammation, which progresses to vessel occlusion. The vessels clinically involved in cardiovascular syphilis serve the aorta and the coronary vessels. Loss of this blood supply results in blood vessel wall death and scar formation.

Scar tissue within the aorta weakens its walls such that the aorta balloons outward, creating an aneurysm. Progression of syphilitic aortic valve damage eventuates in congestive heart failure and possible death. Incomplete syphilitic occlusion of the coronary vessels causes intermittent anginal cardiac chest pain. Myocardial infarction can occur when the coronary vessel occlusion is complete.

8.1.6. Neurosyphilis. Neurosyphilis develops 1–30 years after primary syphilis and involved 5–10% of Oslo's untreated patients.[22] The earliest form of symptomatic neurosyphilis, syphilitic meningitis, is also the least common (10% of neurosyphilis patients). It is manifest by typical meningitis symptoms: fever, headache, photophobia, and stiff neck. The spinal fluid findings are nonspecific—normal sugar and elevated lymphocytes and protein. However, a positive cerebrospinal fluid cardiolipin test differentiates neurosyphilis from other types of aseptic meningitis.

Later manifestations of neurosyphilis are due to blood vessel changes, which consist of vascular inflammation leading to vessel occlusion. The clinical manifestations of vascular neurosyphilis, as with other neurological diseases, depend on which areas of the brain are damaged. Strokes and seizures are early presentations of vascular neurosyphilis. Progression of vascular neurosyphilis consists of the involvement of more and more blood vessels with the subsequent loss of more brain tissue. The associated clinical state is referred to as paresis and is a combination of neurological and psychiatric changes. The latter is primarily a dementing process with loss of intellectual function, although some patients experience grandiose delusions. Common paresis neurological changes are eye pupil abnormalities (Argyll Robertson pupils), slurred speech, and tremors.

Spinal cord neurosyphilis consists of degeneration of the posterior columns. Nerve fibers in these columns transmit deep pain and proprioception sensations. Early clinical manifestations include transient (lasting minutes) sharp pains that radiate into the legs or abdomen. Loss of deep pain sensation in the legs results in repeated painless trauma to the knees and feet. The knees enlarge (Charcot's joints) and ulcers develop on the bottom of the feet (mal perforant).

8.2. Diagnosis

The clinical diagnosis of syphilis is based on its symptoms and signs.[21] The laboratory diagnosis of syphilis is based on visualization of *T. pallidum* and/or demonstration of antibody either to this pathogen or to cardiolipin antigen.

No single finding or test is in itself absolutely diagnostic of syphilis. The most accurate single diagnostic factor is usually the darkfield or direct fluorescent antibody demonstration of *T. pallidum*. The accuracy of these two tests, however, depends on how and where the clinical specimen was obtained, how it was transported to the laboratory, and who performed the test.

Treatment of syphilis is much easier than diagnosis. This infection was one of the first treated with penicillin in the mid-1940s. Unlike many other bacteria, *T. pallidum* has not developed resistance to this antibiotic despite penicillin's being the syphilis treatment of choice for a half-century. A single dose of 2.4 million units of long-acting benzathine penicillin G covers primary, secondary, and latent syphilis of less than 1-year duration. The same penicillin preparation in the same dose also treats both latent syphilis of more than 1-year duration and late syphilis; with these conditions, penicillin is given in three doses, 1 week apart, for a total of 7.2 million units. Some patients with late neurosyphilis do not respond adequately to this benzathine penicillin regimen and require hospitalization for 10 days for treatment with intravenous aqueous crystalline penicillin G or ceftriaxone 2 g given intravenously or intramuscularly daily for 10 days. Some experts give a singly 2.4 million unit dose of IM benzathine penicillin G at the end of the 10-day course to complete a 3-week course of therapy.

Syphilis occurring in pregnancy is treated with the same penicillin regimens. Nonpregnant patients allergic to penicillin can be treated with tetracycline or doxycycline. Pregnant patients with a history of penicillin allergy should have the allergy confirmed by skin tests. Those found not allergic can be treated with penicillin and those with positive skin tests can receive oral desensitization, followed by treatment with standard penicillin doses.

Syphilis treatment, like many other infectious diseases, requires follow-up to assure cure. In addition to resolution of the signs and symptoms of early syphilis, the cardiolipin serological tests should be repeated 6 and 12 months after treatment. A decline of at least two dilutions or a fourfold decline in test titers should occur after treatment, and most tests will revert to negative in cases of P&S syphilis. The anatomic damage occurring in late syphilis cannot be reversed, but treatment will prevent progression of the disease. Management of syphilis patients, as with other STDs, requires evaluation and treatment of sex partners.

9. Control and Prevention

9.1. Epidemiological Basis for Syphilis Control Activities

9.1.1. Transmission Dynamics. Many simultaneous activities are necessary to reduce syphilis, because of the

interactive behavioral and biological dynamics of syphilis spread. The forces responsible for sustaining transmission of any sexually transmitted infection, including syphilis, can be represented in a simple equation, $R = \beta \times C \times D$.[24,25] R represents the reproductive rate of an infection, i.e., the number of new infections produced by an infectious individual. β is the average probability that an infected individual will infect a susceptible partner given exposure. On average, without preventive measures, three of ten sexual encounters between an infectious and uninfected person[26–30] will result in transmission of syphilis (Table 1). C is the average number of new partners exposed by an infectious individual per unit of time. D is the average duration of infectiousness of the specific infection. Public health interventions to prevent syphilis are targeted at reducing the magnitude of β, C, or D by (1) reducing the probability of infecting a susceptible partner; (2) limiting the number of partners who have sex with infectious persons; and (3) reducing the duration of infectiousness.

Most current syphilis control activities emphasize reducing the duration of infectiousness. This approach rests on certain assumptions about the natural history of syphilis (Figure 7): for illustrative purposes, we will assume on the average that the moment of infection is followed by an incubation period of 3 weeks, by a 5-week period of primary syphilis, and by a 6-week period of secondary syphilis, after which the syphilitic infection becomes latent. The 11 weeks of P&S syphilis are considered the period of infectiousness.

Without preventive measures, three of ten sexual encounters between an infectious and an uninfected person will result in transmission of syphilis. Empirical data from national syphilis control programs[31] indicate that an untreated person with syphilis would generate, on average, 0.17 new infections per week during the 5 weeks of primary syphilis and 0.05 new infections per week during the 6 weeks of secondary syphilis, for a reproductive rate of 1.15 (Figure 7). At this level, in the absence of any effective intervention, the infection will propagate in the population. This hypothetical reproductive rate is a weighted average of rates from diverse subpopulations that lie on a spectrum from no transmission (reproductive rate less than 1) to intense transmission (reproductive rate of 2 or more). Although the reproductive rate of 1.15 is probably an underestimate, given slippage in the process of partner notification, it is of heuristic value in considering the impact of an intervention program.

Table 1. Risk of Infection in Persons Exposed to Infectious Syphilis, Selected Studies

Study	Number exposed	Number infected	Percent infected
Alexander et al.[26]	161	100	62.1
Idsoe et al.[27]	77	14	18.1
Moore et al.[28]	79	12	15.9
Schroeter et al.[29]	57	16	30.3
Schober et al.[30]	127	65	51.2

With a control program in which education, clinical diagnosis, and partner notification are effective, syphilis can be treated early in the course of illness and transmission can be interrupted (Figure 8). For example, community education can hopefully motivate infected persons to recognize symptoms of syphilis and seek medical care within 2 weeks after the onset of symptoms. Physicians in the community, if properly trained, can diagnose and treat the disease promptly. A system of partner notification seeks to assure the effective identification, referral, and treatment of persons exposed to infection. Under such circumstances, most of the partners of infectious persons will still be in the incubation period when treated, and the reproductive rate would be lowered to near zero. Such a theoretical goal is unlikely to be achieved. However, given our hypothetical reproductive rate of 1.15, even a 25% reduction in the number of infected partners per case per week during the primary period—from 0.17 to 0.13—would lower the reproductive rate to less than 1 and ultimately interrupt transmission within the community (Figure 8).

This model for controlling syphilis transmission is based on assumptions derived directly from the biological characteristics of *T. pallidum* and the clinical stages of the disease it produces. Compared to other STDs, *T. pallidum* has long division times and, as a consequence, an extended incubation period. Thus, with syphilis, we have a relatively prolonged opportunity to cure the infection before symptoms appear and the host becomes infectious. In addition, *T. pallidum* remains sensitive to long-acting benzathine penicillin. If the organism were to become resistant to this drug and require higher doses or more frequent administration of antibiotic therapy, syphilis control would be compromised. For these reasons, syphilis is a disease for which the interventions of partner notification and prophylactic therapy can have substantial impact. Other STDs with shorter incubation periods (e.g., gonorrhea) or less convenient therapies (e.g., chlamydia) should not be expected to respond as dramatically to control programs as syphilis.[32]

9.1.2. Core Populations. These interventions can be concentrated to achieve maximal impact. Community variability in syphilis reproductive rates will dictate the different levels of public health effort required. Over the past two decades, the heterogeneous distribution of all STDs, including syphilis, has led to the concept of "core populations."[32,33] Core populations (also called "epidemic networks") are defined as those sociosexual networks that contribute a disproportionate share to sustaining STD rates

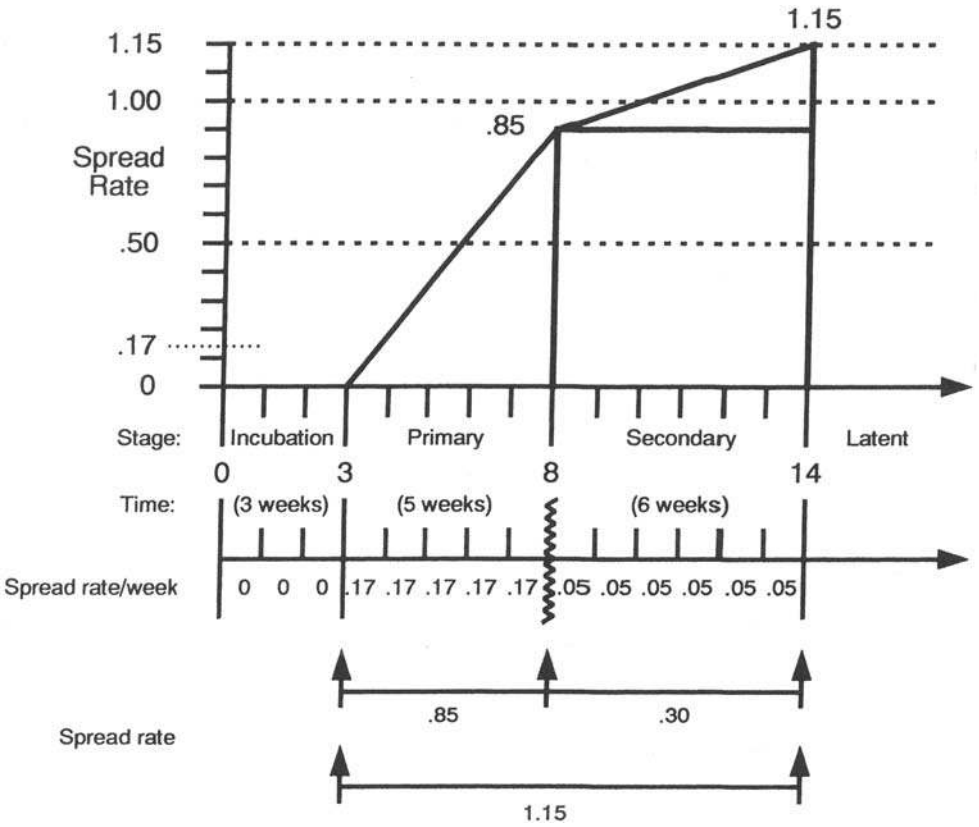


Figure 7. Projected reproductive rate of syphilis.

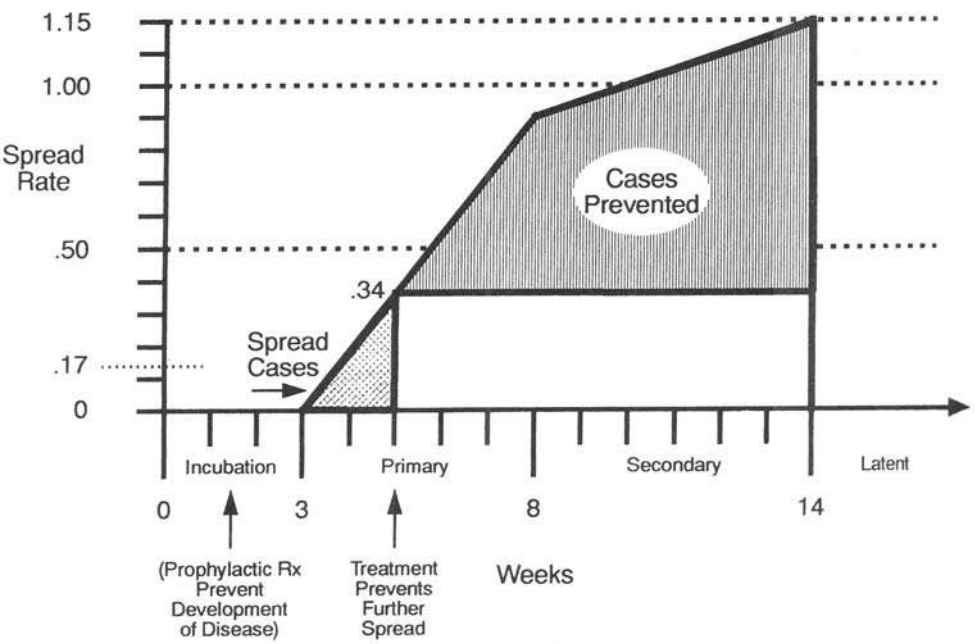


Figure 8. Preventing syphilis by treatment.

in communities. From the above model, as well as empiric data,[27] syphilis core groups will need higher rates of sex partner change than other STDs to maintain the infection within a sexual network.

Evidence supports this epidemic network concept for syphilis. In Seville, Spain,[34] syphilis (along with gonorrhea and chlamydia) showed a distinct spatial clustering. In upstate New York,[35] the distribution of syphilis was more diverse than gonorrhea, but still could be connected through two endemic foci: (1) inner-city, heterosexual African-American populations and (2) suburban, homosexual/bisexual white and African-American groups. In Multnomah County, Oregon,[36] a syphilis core population was identified using geographic and sociodemographic measures as proxies for these sociosexual networks. The Oregon core group, like others, was characterized by spatial clustering, low socioeconomic status, and a high proportion of African-American persons. In British Columbia, Canada, street nurses successfully used social networking approaches, environmental observation, peer outreach, serial interviewing, testing, and treatment in the field to increase the proportion of syphilis cases identified in a recent heterosexual outbreak.[37]

9.2. Control and Prevention

A recurrent theme in the control and prevention of syphilis is the need for a multidisciplinary coordinated, multifocal program. A variety of interventions, tailored to local epidemiological and social circumstances, are required to reach the epidemic networks and to lower the reproductive rate. The following sections summarize the chief elements of a coherent syphilis control program.

9.2.1. Screening. The advent of serological detection methods provided the technological impetus for modern syphilis control programs. Widespread testing to eliminate syphilis has enormous intuitive appeal. Nonetheless, the zeal for mass screening has been tempered by realistic comparisons of screening costs.[38] Such considerations have produced general agreement that universal syphilis screening in many industrialized nations, including the United States, cannot be justified.

Less unanimity exists, however, about the value of more targeted screening. First, attitudes toward syphilis screening change drastically with the slope of the trend line. The subjective valuation of screening a particular population will change if that community's perceived risk of transmission is thought to be high (e.g., the epidemiological importance of screening commercial sex workers). Second, confusion exists between the role of screening as a tool for case finding and the role of screening in interrupting transmission. Case finding generally rests on a subjective judgment of the social value of finding an infected person (e.g., the use of universal prenatal screening to detect the rare case of congenital syphilis). The interruption of transmission through syphilis screening is harder to verify primarily because detection of asymptomatic cases cannot be directly equated with diminished disease transmission. Except in situations in which syphilis incidence is increasing, most screening programs will identify persons in noninfectious stages of the disease, and thus will have little impact on transmission.

As described previously, the concept of screening in "core populations" has become increasingly popular. Whether these groups are defined by occupation (e.g., commercial sex workers), by facility (e.g., prisons, hospital emergency rooms), by risk environment (e.g., gay bathhouse, "crack house"), by geographic location (e.g., census tract), or by sociodemographic variables (e.g., poverty, ethnicity), the epidemiological principle is the same. Screening targeted to high prevalence areas should interrupt core transmission and should have an accelerated impact on reducing community incidence. This approach has been evaluated in Oregon, using geographic and sociodemographic markers for the core.[36] The results of this screening intervention were inconclusive; new cases were identified, but their connection to true core transmitters was unclear.

No incontrovertible guidance emerges from the literature on experience with syphilis screening. Despite the development of better quantitative tools for evaluation of costs and benefits, considerable subjective judgment is still required. Only one published report directly addressed the question of whether or not screening interrupts transmission of syphilis,[39] though the size and scope of that study may not have been sufficient to warrant generalization. Most studies discuss the "yield" of active syphilis cases; even similar syphilis yields evoke markedly different value judgments regarding public health utility.

Nevertheless, the diversity of screening data (and opinions) suggests two common themes. First, wide variation in the prevalence of syphilis provides a justification for screening as a form of cross-sectional syphilis surveillance. Second, the observed variation in syphilis positivity means that screening decisions should be based on local data—whatever criterion for efficacy is invoked. If screening is to have any value as a mechanism for case finding or for disease control, it must be locally applied and locally analyzed.

9.2.2. Clinical Diagnosis as a Tool for Disease Control. Syphilis symptoms and signs are protean. Clinical diagnosis, while difficult, is crucial for both case finding and prevention. Moreover, clinical services are the final

common pathway through which treatment is given, cure is achieved, and prevention is accelerated. Thus, these services must be easily accessible to the core populations.

Few studies have documented either the percentage of infected patients who recognize their syphilis symptoms or the percentage of those with recognized symptoms who seek medical services. Without such recognition and health-care seeking, opportunities for clinical diagnosis are diminished. In one nationwide study, 45% of persons who were identified through partner notification efforts admitted that they had recognized symptoms of syphilis but did not seek medical advice.[3]

Despite these difficulties, the majority of early syphilis cases reported to public health authorities have resulted from clinical diagnoses based on patient symptoms. In the late 1940s, about two-thirds of all persons diagnosed with primary or secondary syphilis sought medical care on their own initiative.[40] Likewise, in 1990, 62% of those with early syphilis obtained clinical services based on their own suspicions of illness (CDC, Division of STD/HIV Prevention, unpublished data, July 1991).

Even when the symptoms of genital ulcers occur and the patient seeks care, diagnosis using clinical parameters alone is notoriously difficult. This situation is made even worse when low prevalence leads to clinician unfamiliarity. For example, in one series of 100 patients with genital ulcers, clinicians without benefit of laboratory findings were unable to identify the specific microbial etiology more than half the time.[40]

The current evolving epidemiology of incident syphilis, changing risk groups, and social environments present challenges for elimination and STD program activities. Syphilis is increasingly diagnosed in the private sector, and concerns regarding immediate recognition of incident infection, as well as case reporting, are increasing. Public health services face increasing pressures from increasing demand and decreasing financial resources. The social contexts of poverty, homophobia, racism, and socioeconomic discrimination continue to drive the concentration of syphilis in those with high-risk sexual behaviors, poor access to care, or both.[8]

9.2.3. The Role of Treatment. Providing proper treatment for both symptomatic and asymptomatic persons is the sine qua non of syphilis control. Penicillin treatment based on presumptive diagnosis of syphilis is inexpensive, simple, safe, and effective. Thus, as shown on the model above, early and adequate treatment of syphilis patients and their sex partners is the essential means of preventing community spread.

In addition to treating those diagnosed with syphilis, selective prophylactic (preventive or epidemiological) treatment also has a major role in syphilis control strategies.[41] Over the years, this approach of selective preventive treatment has helped to limit multiple outbreaks of syphilis. Coupled with the process of active partner notification (see Section 9.2.4), prophylactic treatment of persons exposed to infectious syphilis has effectively controlled epidemics in both rural[42] and urban[43] settings. Benzathine penicillin for syphilis is especially helpful for outbreaks. Because it produces treponemicidal concentrations of penicillin for at least 3 weeks, this agent provides an interval of "antibiotic" quarantine[44] during which reinfection is hindered.

Within certain circumscribed communities with a high prevalence of infection, selective mass penicillin treatment has been useful. For example, in Fresno, California, in 1977, selective mass treatment of commercial sex workers interrupted an ongoing syphilis epidemic.[40] The same strategy, though for different indications, has been applied by the World Health Organization for eradicating yaws.[45,46] Not surprisingly, this mass treatment approach also lowered the prevalence, albeit temporarily, of venereal syphilis in those communities where it was applied.

Azithromycin, 2 g in an oral single dose, would be an ideal alternative to the penicillin injection, since it could easily be given in field settings. It has been reported as effective as the IM penicillin therapy in a treatment trial conducted in Mbeya, Tanzania.[47] However, recent reports of azithromycin-resistant *T. pallidum* in the United States, Ireland,[48] and Canada[49] indicate the importance of continued monitoring for resistance.

9.2.4. Partner Notification. Rapid investigation of sex partners is aimed at bringing persons with early syphilis to treatment before they can spread the infection within the community. In fact, syphilis is the prototypic STD on which the prevention strategy of partner notification was based, both in theoretical concept and in practical application.

Traditionally, syphilis control programs in the developed countries have emphasized active intervention by health providers to interview patients, to identify sex partners, and to assure that partners are evaluated and treated.[50] The privacy of patients and partners is rigorously protected. Up through the 1960s, a number of reports attested to the success of this epidemiological approach for syphilis control.[28,51–55] Whether applied to isolated rural communities,[56,57] selected urban neighborhoods,[58] or captive prison settings,[50] rapid sex partner identification, location, and treatment reduced syphilis occurrence.

The partner notification process involves a series of interrelated activities: interviewing the infected patient to obtain a complete list of exposed sex partners, finding these

persons in the community, and assuring that they are treated promptly. In the United States, interviewing techniques have been developed to encourage patient cooperation in naming a maximum number of partners.[3] However, during the 1960s and 1970s, the contact index—a measure of the yield of the syphilis interview—steadily declined (Figure 9). Possible interactive reasons for this trend include a true decrease in the number of sex partners in infected persons; changing environments for sexual relations whereby a greater percentage of partners are anonymous (e.g., gay bathhouses, crack houses); an increasing atmosphere of distrust of all governmental organizations, including those delivering public health services; deteriorating interviewing techniques; and the increasing competition for partner notification services of other STDs.

Using specific populations, rather than infected individuals, as the units for syphilis intervention is a means for overcoming problems with naming and locating sex partners. In the past, this community-oriented approach was a type of "cluster" interviewing. Syphilis patients, in addition to their known sex partners, were queried about other persons in whom and specific social settings in which syphilis transmission may have occurred.[60] Although this cluster approach produced a lower yield of infected, previously untreated patients than following up those specifically named as sex partners, it still identified a relatively high percentage of persons who might not have been reached in any other way. In fact, depending on how the "socially related group" was defined, up to one in five people examined had untreated syphilis.[60]

In recent years, similar population-based approaches have been used to identify particular communities at high risk of syphilis. Examples of situations in which this community intervention was successful in stemming syphilis outbreaks include (1) a Native American tribe reluctant to name any partners to outside officials,[61] (2) a Philadelphia neighborhood where crack-cocaine environments led to unsafe sexual behaviors and high levels of syphilis,[62] and (3) a Montgomery County, Alabama, intensive program that identified sociosexual networks responsible for the continued spread of syphilis.[63]

The Internet is a rapidly growing venue for the acquisition of sex partners.[64] Recent published reports describe the use of the Internet by MSM to meet multiple casual sex partners and partners of unknown HIV status. This is a growing public health concern since some Internet users with syphilis report unprotected anal intercourse, and have difficulty identifying partners or have anonymous partners. Consequently, public health officials are developing strategies to use the Internet to reduce transmission of syphilis and other STDs by

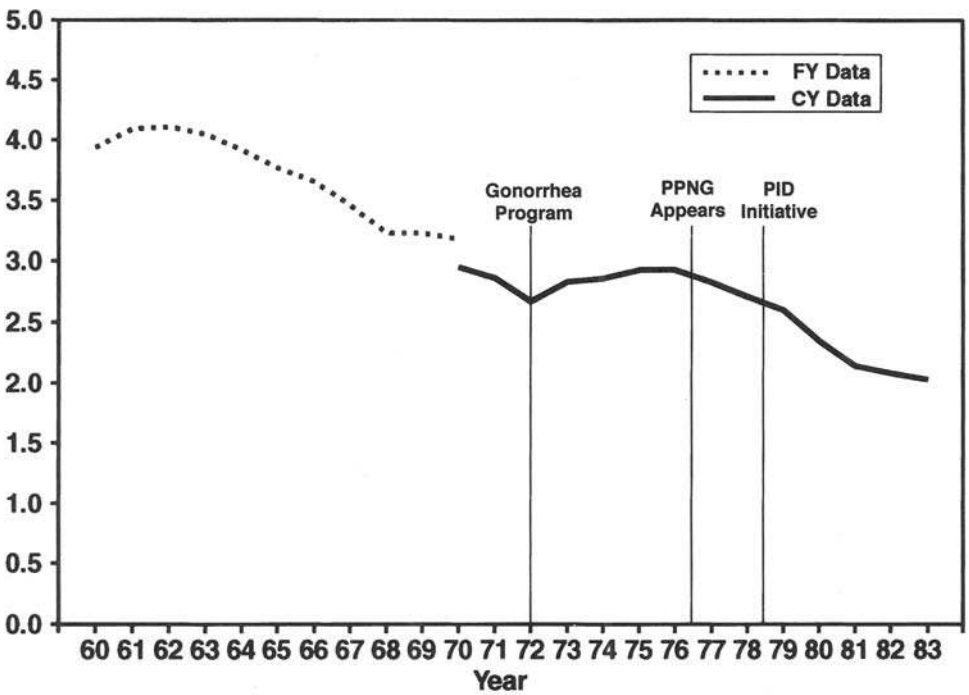


Figure 9. Contact index, 1960–1983, primary and secondary syphilis, public STD clinic patients.

notifying partners through screen names,[64] providing health education and prevention messages on Web sites via pop-up ads and links to Web sites that offer information on STD testing sites, making health education available in chat rooms to answer health-related questions, and offering online test result reporting.[65]

9.2.5. Health Education. Our current community health education efforts usually encourage primary prevention (preventing acquisition of syphilis) in persons at risk. Over the last two decades, HIV prevention resources have stimulated community education for safer sexual behaviors, which in turn has led to safer social norms. Health education interventions can also be directed to individual patients diagnosed with syphilis. They are designed to promote early therapy if symptoms such as genital ulcers recur, avoidance of sex after onset of syphilis symptoms, and notification of all sex partners. If successful, this counseling will result in both primary prevention (prevention of further syphilis transmission) and secondary prevention (prevention of later complications).

Other primary prevention approaches, financed by HIV prevention dollars, could also help reduce syphilis. These include both advocating risk avoidance methods and distributing condoms and counseling about safer sex. Condoms, although shown to be effective in limiting the transmission of HIV and other STDs,[66] are not used regularly by many high-risk individuals. Thus, we need more creative methods of condom promotion, including making them more widely available at little or no cost, to enhance syphilis control. However, sexual behavior change is not easily achieved in populations characterized by drug use, poverty, low education, unemployment, and inadequate health care. These groups need to be specifically targeted by health education campaigns and provided improved access to health care for syphilis screening, diagnosis, and treatment.[67]

Experience with HIV community education has shown that (1) changing social norms is fundamental to influencing safer individual sexual behavior and (2) reinforcing these safer behaviors regularly by trusted peers is crucial to preventing relapse to unsafe practices. Syphilis control in the homosexual community has already benefited from these HIV lessons. In socially disadvantaged communities, pilot programs using commercial sex workers as outreach workers have been successful in educating high-risk women about syphilis and in identifying pregnant women who need prenatal care, including syphilis testing.

9.2.6. Immunization. Another hope for primary prevention of syphilis is active immunization against either the infection or the disease. Because of the immune response generated during the natural history of untreated *T. pallidum* infection, prospects for developing a protective vaccine might seem encouraging. Acquired resistance to further *T. pallidum* infection occurs from both human and experimental syphilis.[68] However, attempts to generate long-term protection using a single immunogen have been disappointing.[69] With genome libraries and sequence now available, we might explore the rare *T. pallidum* outer membrane proteins, which are the first targets of the immune response. Their discovery and use in a multivalent vaccine may lead to the production of an effective vaccine for the future.[70]

10. Unresolved Problems

1. *More accessible clinical services*: All symptomatic patients must be able to receive prompt diagnosis and treatment. If public or private facilities are not available or are overburdened in the high-incidence communities, then temporary, mobile alternatives must be provided.
2. *Population-based partner notification*: Data obtained from the process of eliciting the location and sociosexual networks of sex partners must be used for both individual and population benefits. Where epidemiological analysis of partner patterns reveals high-risk communities, public health officials must move quickly to intervene clinically.
3. *Wider prophylactic treatment*: Among selected population for which levels of infectious syphilis are high, prophylactic treatment based on "epidemiological" indications must be considered. Alternatively, targeted community screening, with immediate presumptive treatment of all rapid plasma reagin-positive patients, must be implemented.
4. *Greater community involvement in syphilis control*: Public health officials must continue to build on the network of community-based organizations generated by interest in HIV prevention. Local decision makers must be reeducated about the importance of syphilis and the role of early diagnosis/treatment in preventing its spread, so they can become advocates for promoting behavioral change and securing clinical resources.
5. *Ongoing provider training*: Clinicians must be trained to "think syphilis," especially if practicing in high-incidence communities. Residency programs should include rotations through local STD clinics, not only to upgrade clinical STD skills but also to allow an understanding of real-world behaviors contributing to syphilis spread.

In actuality, these challenges are really nothing new.[6] "Environmental" or "community-based" approaches to controlling syphilis have long been with us. In multiple situations during the past four decades, after intensive screening programs documented high levels of syphilis in particular sociosexual networks, the "risk marker" of being in that network became enough of an epidemiological justification to "presumptively treat" persons for syphilis.[6] Not surprisingly, the morbidity level in the community was drastically lowered.

Are we simply suggesting reinventing the wheel? In one sense, yes; in another sense, no. Bolstered by our rapidly evolving electronic data system, we are better able today to target interventions, design appropriate evaluations, and disseminate the results of our efforts than we were in earlier years. Working together, epidemiologists (clinical, behavioral, and statistical) and program managers need to merge their skills both to document the success of specific syphilis control activities and to refine those that are less effective. This epidemiological/managerial interaction to continually evaluate our interventions will keep syphilis control at the cutting edge of public health well throughout the 21st century.[6,8]

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CHAPTER 37

Nonvenereal Treponematoses

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1. Introduction

The endemic treponematoses are a group of chronic diseases affecting primarily the skin of children and young adults who live in remote, impoverished areas between the tropics of Cancer and Capricorn. They are considered as a group because they have many clinical, pathological, and epidemiological features in common (see Table 1). They are also caused by spirochetes that are closely related to one another and to *Treponema pallidum* subsp. *pallidum* of venereal syphilis.[1] These diseases with their etiologic treponeme in order of their worldwide prevalence are yaws (*T. pallidum* subsp. *pertenue*), endemic syphilis (*T. pallidum* subsp. *endemicum*), and pinta (*Treponema carateum*).[2] Other related treponemes cause venereal syphilis in rabbits (*Treponema cuniculi*) and asymptomatic infections in African baboons (*Treponema freiborgblanc*), but they have no epidemiological significance as far as human infections are concerned.

2. Historical Background

The endemic treponematoses are ancient diseases. Yaws, for example, is thought to have its origins in Africa during the middle Pleistocene period (1.5 million years ago), with characteristic bony lesions having been found in skeletal remains of *Homo erectus* in Nairobi.[3] There is an ongoing debate about the origins of the treponematoses. In the early 1960s Hudson and Hackett proposed competing theories. Hudson argued that the causative organisms were genetically identical, and that the differing clinical manifestations were solely due to the distinct climates of endemic regions.[4] Hackett's position was that while closely related, the organisms were genetically distinct, and that this would account for different disease manifestations.[5] The latter position seems to be the more tenable given the organisms' distinct growth characteristics in experimental animals,[6] lack of complete cross-immunity across subspecies (as evidenced in the rabbit model),[7, 8] and recently discovered genetic differences between *T. pallidum* subsp. *pallidum* and the nonvenereal subspecies.[9–11]

Until the last few decades the endemic treponematoses were a major cause of morbidity in developing nations, and yaws in particular was one of the most prevalent infectious diseases of the tropics before the penicillin era. In 1950, an estimated 70–100 million people living in large areas of Africa and Southeast Asia, the western Pacific islands, and parts of the Americas had yaws; another 1 million living in the Middle East, the Balkans, and North and West Africa had endemic syphilis; and 700,000, primarily American Indians of Central and South America, had pinta (see Figure 1).[12] The ratio of infectious to latent yaws was 1:3 or more, and most infections occurred in children under 15 years of age.

The first drugs used effectively against the endemic treponematoses (and venereal syphilis) were not penicillins, but rather arsenical compounds, discovered in 1910. In the 1920s and 1930s many developing countries organized yaws treatment campaigns using multiple injections of arsenicals at hospitals and by mobile units and rural dispensaries, with resounding clinical success.[6] These early yaws campaigns were a launching point in the development of primary national healthcare systems in many of the involved

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Table 1. Major Features of the Treponematoses

	Venereal syphilis	Endemic syphilis	Yaws	Pinta
	T. pallidum subsp. *pallidum*	*T. pallidum* subsp. *endemicum*	*T. pallidum* subsp. *pertenue*	*T. carateum*
Age of infection	15–40	2–10	5–15	10–30
Occurrence	Worldwide	Africa, Middle East	Africa, South America, Oceania, Asia	Central and South America
Climate	All	Dry, arid	Warm, humid	Warm
Transmission				
Direct				
Venereal	Common	Rare	No	No
Nonvenereal	Rare	Common	Common	Probable
Congenital	Yes	Unproven	No	No
Indirect				
Contaminated utensils	Rare	Common	Rare	No
Insects	No	No	Possible	No
Reservoir of infection	Adults	Infectious and latent cases	Infectious and latent cases	Infectious cases
Ratio of infectious to latent cases	1:3	1:2	1:3–5	?
Late complications	+	+	+	+
Skin, bone, cartilage	+	+	+	No
Neurologic	+	Unproven	No	No
Cardiovascular	+	Unproven	No	No

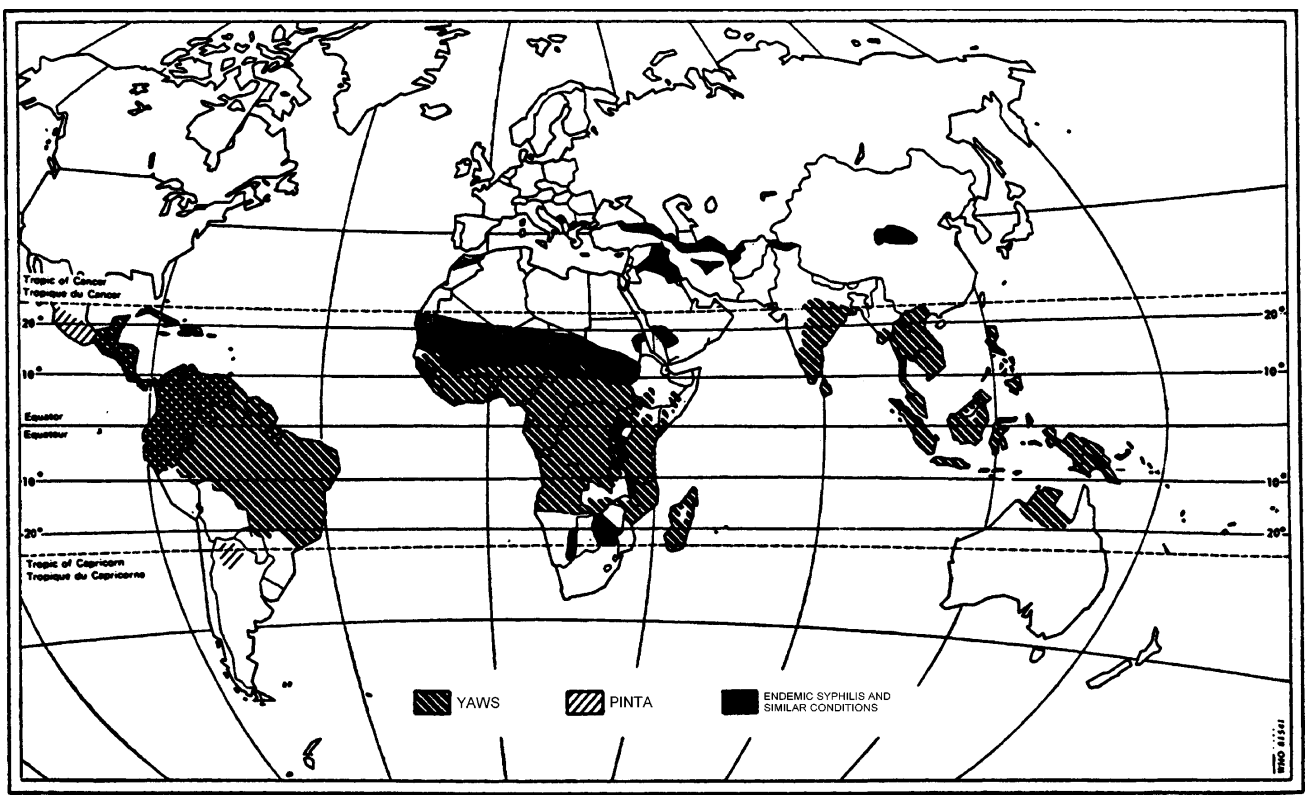


Figure 1. Geographic distribution of the endemic treponematoses in the early 1950s.

countries.[13] Importantly, these first campaigns focused solely on clinical (not latent) cases, and thus made only a dent in the total number of yaws cases.

The development of long-acting penicillin preparations, producing a treponemicidal blood level for 3–4 weeks after a single injection, made mass treatment of treponemal infections much more feasible.[14] In 1949, the nascent World Health Organization (WHO) agreed to conduct coordinated mass treatment campaigns against yaws, endemic syphilis, and pinta. Haiti and the Dominican Republic were chosen as the sites for pilot projects, and the initial campaigns confirmed the efficacy of single injections of penicillin aluminum monostearate in mass treatment, the need to examine at least 90% of the target population during the initial survey, and the need to conduct re-surveys of treated populations to treat new cases and re-infections.[14] With logistical support from the United Nations International Children's Emergency Fund (UNICEF), the WHO extended the program to 46 countries, and from 1952 to 1968, an estimated 160 million persons worldwide were examined during initial surveys, an additional 300 million examined during re-surveys, and 50 million persons with clinical or latent infections and their contacts were treated with penicillin. Overall endemic treponematosis disease prevalence was reduced by 95%,[14,15] and at the end of these campaigns the endemic treponematoses appeared to be on their way to extinction, especially in view of the improvements in living standards that occurred in many of the endemic areas.

By the beginning of the 1970s responsibility for controlling and eradicating remaining cases was transferred to individual national and local governments, and the vertical mass treatment approach was switched to one integrated into the basic health services system. This approach was far less effective, and by the end of the decade many formerly endemic areas saw a resurgence in endemic treponematoses cases, especially among children and young adults born after penicillin treatment campaigns were completed. By the early 1980s the number of persons infected with the endemic treponematoses was estimated at 2.5 million,[6] and under WHO recommendation several countries reinstituted mass treatment campaigns, with varying success.

3. Methodology

Until the late 1980s current epidemiological data on the endemic treponematoses were available from the regional offices of the WHO and its *Weekly Epidemiological Record*[16] and the proceedings of yaws symposia.[17–19] Over the last 15 years most national governments have ceased collecting epidemiological data on the endemic treponematoses, and neither yaws, endemic syphilis, nor pinta is a disease that member states must report to the WHO. The most recent estimates come from the 1998 WHO World Health Report and from expert analysis of several recent articles in the medical literature that focus on surveys in particular communities.

3.1. Sources of Mortality Data

There is little pathological or epidemiological evidence that the endemic treponematoses acquired in childhood subsequently cause fatal late cardiovascular or central nervous system (CNS) complications usually associated with venereal syphilis. Where venereal syphilis is prevalent together with yaws or endemic syphilis, however, it is impossible to differentiate visceral, ocular, and neurological lesions caused by venereal syphilis from those that might result from yaws or endemic syphilis.[20,21]

3.2. Sources of Morbidity Data

The WHO, through analysis of data reported to its regional offices, reported its latest estimates in its 1998 World Health Report. Several more recent articles in the medical literature provide data on trends in particular endemic areas.

3.3. Surveys

Clinical and seroprevalence surveys for yaws and endemic syphilis were conducted by many African and Asian countries in the 1980s as part of a WHO initiative to control the resurgence of these diseases. Since then, individual countries have sporadically carried out surveys in areas of high prevalence.

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organisms. The pathogenic treponemes cannot be cultured *in vitro,* but their identification by dark-field microscopy of lesion exudate provides instant diagnosis. This approach is, however, becoming less common as it takes trained and experienced staff to properly identify the pathogenic treponemes. A more sensitive and specific[6] method is direct fluorescent antibody (DFA) identification of treponemes on a slide of lesion exudate, done at a reference laboratory.

3.4.2. Serological and Diagnostic Methods. Serological tests routinely employed in seroprevalence surveys are the venereal disease research laboratory (VDRL) and

rapid plasma reagin (RPR) tests.[22] These tests are not specific, but their reactivity and titer in an appropriate clinical setting give a good idea of the prevalence of latent treponemal infection. Specific treponemal antibody tests (e.g., *Treponema pallidum* hemagglutination test and fluorescent treponemal antibody absorption test) can be used to confirm a diagnosis, but these antibodies stay positive for many years after infection, even if successfully treated. No serological or microscopic test to date can distinguish between *T. pallidum* subspecies or between *T. pallidum* and *T. carateum*.[23]

4. Biological Characteristics of the Organism

The members of the genus *Treponema* fall under the Spirochaetaceae family which also contains the *Borrelia* species. The four treponemes pathogenic in humans cause clinically distinct diseases, and for this reason they were initially classified as four distinct species. Subsequent DNA hybridization studies caused a reclassification into the current scheme, in which three of the treponemes are subspecies of *T. pallidum*: *T. pallidum* subsp. *pallidum*, *T. pallidum* subsp. *endemicum*, and *T. pallidum* subsp. *pertenue*.[1,2] The fourth treponeme, *T. carateum*, has been impossible to isolate for comparative genetic studies,[6] and so it continues to be classified as a separate species. *Treponema pallidum* and its subspecies are obligate anaerobes. Difficulties in culturing these organisms in a cell-free *in vitro* system have limited comparative studies of their genetic and antigenic structure, but recent experimenters have used immunoblot techniques, specific monoclonal antibodies, and synthetic peptides to look for antigenic differences, so far without success.[6] Prototype strains of yaws and endemic syphilis treponemes produce lesions in only a few experimental animals. The lesions produced in these animals are similar to those occurring during the early stages of infection in man. Subtle differences in virulence and lesion morphology exist between subspecies and strains of *T. pallidum endemicum* and *pertenue*. Similar subspecies and strain differences exist in most species of pathogenic bacteria.

The pathogenic treponemes are exquisitely sensitive to penicillin. Their multiplication by binary fission takes an estimated 32 h to complete[24] and is the reason why long-acting penicillin preparations that produce low but persistent penicillin blood concentrations for 3–4 weeks are recommended for therapy. Importantly, in the past decade there have been a few reports of penicillin treatment failures in Papua New Guinea[25] and Ecuador,[26] a trend that could become very problematic if resistance grows.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Since most national governments have stopped collecting epidemiological data on the endemic treponematoses, the current number of cases is unknown. The latest figures from the WHO in its 1998 World Health Report put the estimated worldwide prevalence at 2.5 million, with an annual incidence of 460,000 cases.[27] However, a high percentage of latent cases and the prevalence of "attenuated," less readily diagnosable, yaws and endemic syphilis in areas of reduced transmission[28] may mean that the actual numbers are even higher. The large majority of current endemic treponematoses cases are yaws, with yaws-endemic foci remaining in West and Central Africa, Southeast Asia, on some of the Pacific islands including Papua New Guinea, Central America, and northwest South America.[6] In certain African, Asian, and South American countries, prevalence levels are thought to approximate the pre-penicillin campaign levels.[23] Endemic syphilis continues to be seen in high numbers among nomadic and semi-nomadic tribes in the Middle East (especially Saudi Arabia) and in Sahel Africa.[29,23] Treatment campaigns and improving standards of living have dramatically decreased the prevalence of pinta, and it is now believed that the disease exists only in a number of isolated, rural, indigenous communities in Mexico and South America, especially in parts of the Brazilian Amazon rain forest.[30]

5.2. Epidemic Behavior and Contagiousness

Yaws is highly contagious and can spread rapidly in susceptible populations, such as rural communities with poor hygiene, minimal clothing, and limited access to medical services.[6] Endemic syphilis tends to affect isolated, nomadic populations, and its epidemic potential is less than that of yaws. Indeed, the prevalence of endemic syphilis has decreased because of small improvements in the living standards and personal hygiene of many populations during the last century and a half, even before the advent of modern treatment.[6] In contrast to the other endemic treponematoses, pinta is very difficult to transmit by person-to-person contact and has little epidemic potential.

5.3. Geographic Distribution

The worldwide distribution of the endemic treponematoses in the mid-1980s is shown in Figure 2. Humid, rural, tropical areas of the world, particularly throughout Africa,

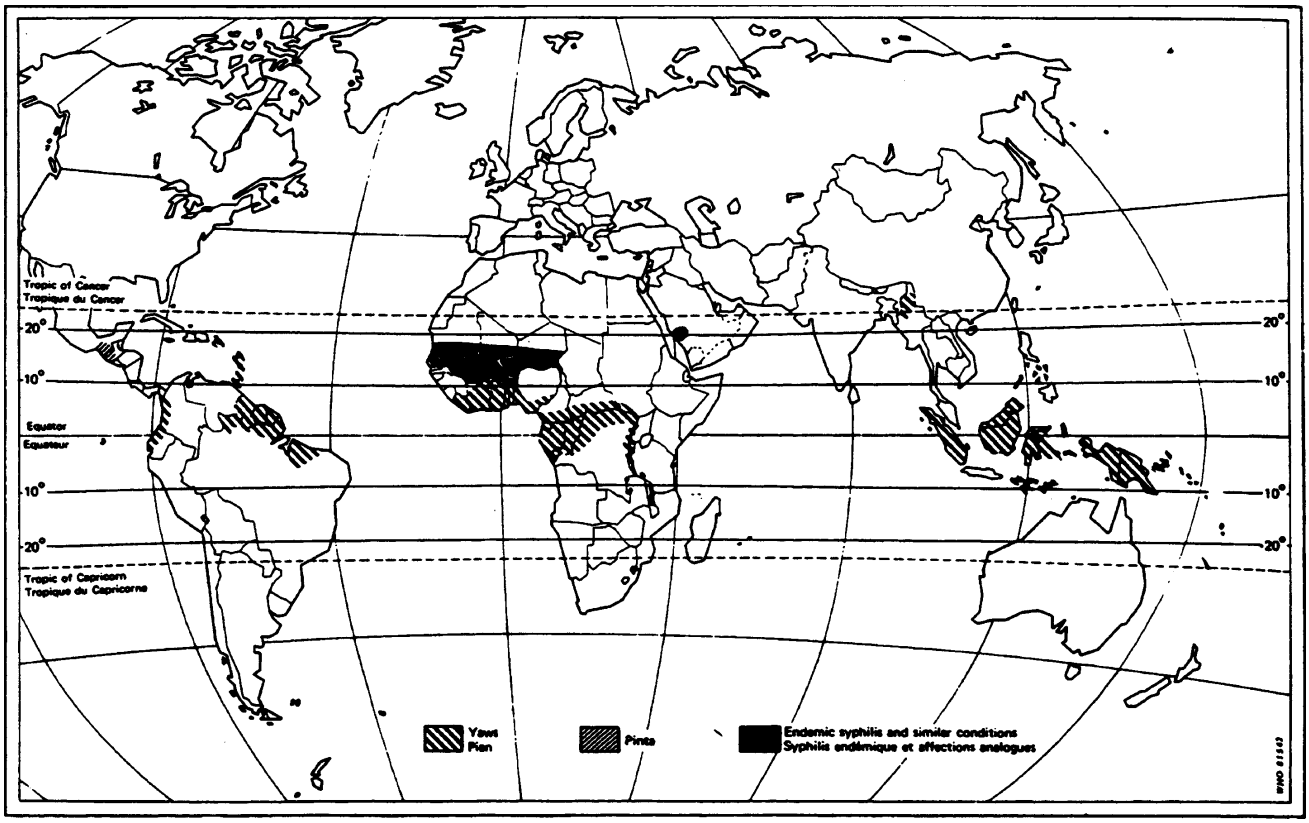


Figure 2. Geographic distribution of the endemic treponematoses in the 1980s.

are hosts to yaws, whereas endemic syphilis exists mainly in dry, arid regions of the eastern hemisphere, such as Saudi Arabia and the Sahel countries of Africa.[28] Pinta, once endemic to Central and South America, was thought to have been extinct (with the exception of remote areas of the Amazon rain forests) since 1975.[31] However, a remote case of pinta diagnosed in a woman who was visiting Austria (but who had lived in Cuba for 7 years) indicates that the disease may in fact still be prevalent in once-endemic areas.[32]

5.4. Temporal Distribution

There is no seasonality in occurrence of the endemic treponematoses, but the number, morphology, and infectiousness of cutaneous yaws lesions change during the wet and dry seasons in Africa and Asia. The high humidity associated with seasonal rains promotes an exuberant growth of papillomas and the survival of treponemes in their serous exudate, thus increasing infectiousness and the probability of person-to-person transmission. The skin is also softened by high humidity and is more susceptible to abrasions and lacerations, which are a portal of entry for treponemes. Lesions become less numerous during the dry seasons, and the papillomas tend to occur mainly in moist areas of the body, such as the axilla and groin.[6].

5.5. Age

In endemic areas, yaws is usually acquired before 15 years from infected siblings or playmates, and active early lesions are found almost exclusively in this age group. Endemic syphilis tends to occur in family groups, with the infection being acquired first by children, who then spread it to susceptible adults. Pinta usually first appears in susceptible adults.

5.6. Sex

Pinta tends to occur more frequently in men, but there is no sexual predilection for yaws or endemic syphilis.

5.7. Race

Race is not a factor.

5.8. Occupation

The diseases have no relationship to occupation per se, but the vast majority of those affected live in communities that survive by subsistence farming.

5.9. Occurrence in Different Settings

These treponematoses are usually found within families and among playmates.

5.10. Socioeconomic Factors

The endemic treponematoses are prevalent today in impoverished, remote, and rural populations lacking adequate clothing, water, sanitation, and medical care.

6. Mechanisms and Routes of Transmission

Transmission of yaws occurs by direct skin-to-skin contact, particularly by breaks in the skin barrier. Fingers contaminated with fresh lesion exudate readily transmit the viable treponemes from one individual to another. Various species of flies, particularly nonbiting flies (*Hippelates pallipes*), have also been implicated in the transmission of yaws.[6]. However, the efficiency of such transmission by flies is probably epidemiologically insignificant.

Endemic syphilis can be transmitted directly by person-to-person contact or indirectly by sharing of drinking cups contaminated with treponemes present in the serous exudates from lesions on the oral mucosa or at the corners of the mouth.

Transmission of yaws or endemic syphilis can occur during sexual intercourse, providing one partner has infectious lesions in the genital area, and the other is susceptible to infection. Unlike venereal syphilis, however, congenital transmission of yaws or endemic syphilis has not been convincingly documented. Indeed, experiments in the guinea-pig model[33] demonstrated a lack of transplacental transmission of *T. pallidum* subsp. *pertenue*.

The route of transmission of pinta is not clearly elucidated.[28] Most likely, transmission occurs through prolonged intimate contact with an adult with active disease. Therefore, household members of an infected individual are more likely to acquire the disease. However, infection rates are low, even between married couples.

7. Pathogenesis and Immunity

The endemic treponematoses manifest histopathological changes and serological responses that closely resemble those of venereal syphilis. The only practical means of comparing their pathogenicity is by experimental animal infections. *Treponema pallidum* spp. *pallidum* is more invasive and pathogenic than *T. pallidum* spp. *pertenue*. *Treponema carateum* is pathogenic only for man and higher primates.

Like venereal syphilis, infection with nonvenereal treponematoses produces localized lesions. In particular, a granuloma is the prototypical pathological lesion of both yaws and endemic syphilis. This reflects a strong, delayed hypersensitivity response to the presence of treponemes in skin and bone. Throughout the course of the disease, normal skin and bone are replaced by scar tissue, causing disfigurement and occasional disability. Unlike venereal syphilis, however, cardiovascular, neurological, and visceral lesions are extremely rare.[20]

Protective immunity waxes and wanes during the course of untreated yaws and endemic syphilis, especially during the 5 years following initial infection. In studies performed by Turner and Hollander,[8] this immunity was shown to be specific to the treponemal subspecies. Later experiments performed in the hamster model[34] showed that immunity against re-infection with the homologous organism was not absolute among different *T. pallidum* species. This is manifested by the reappearance of infectious cutaneous lesions, and is the reason why penicillin must be given to asymptomatic persons living in highly endemic communities who are very likely to have latent infections.

8. Patterns of Host Response

The manifestations of yaws and endemic syphilis are usually mild, with lesions restricted to the skin and mucous membranes. The clinical features occur in both early (which includes primary and secondary lesions) and late stages.[28] The lesions usually heal spontaneously after several months, but can result in more aggressive disease with destruction of skin and bone. Those who suffer severe disease may be genetically predisposed.

In areas where infection with the nonvenereal treponematoses are endemic, serological surveys invariably detect more individuals with latent infections than those with clinically manifested disease. Many of these patients have no recollection of early disease manifestations, suggesting that asymptomatic infection is frequent or that their earlier lesions were inconsequential or mistakenly interpreted as something else.

8.1. Clinical Features

The incubation periods of yaws, endemic syphilis, and pinta are directly related to the number of treponemes in the

inoculum. The initial lesion(s) appears at the portal of entry after an incubation period of 9–90 days.

Pinta, yaws, and endemic and venereal syphilis are chronic infections with early and late clinical manifestations. Early lesions are infectious and usually are not destructive, whereas late lesions are not infectious but are destructive.

8.1.1. Pinta. Initially, one or more painless, papular lesions form on the upper or lower extremities, face, or other exposed areas. Individual lesions expand to form irregular, reddish, or bluish patches 5–10 cm in diameter with raised borders. Several weeks or months later, disseminated lesions ("pintides") with a morphology similar to that of the initial lesion appear on different parts of the body. Hyperkeratosis with desquamation, itching, and formation of fissures within the borders of lesions is common and may be accompanied by lymph node enlargement. The skin is the only organ pathologically affected.

In the late stage, the involved skin becomes atrophic, and the bluish coloration of the initial lesion caused by accumulation of melanin pigment in the dermis fades. This leaves the patient disfigured by red, blue, and white spots on the face, neck, arms, hands, legs, and feet. Eventually the lesions cause sclerosis of the dermis and depigmentation, leaving scars that resemble vitiligo. The evolution from the time of the appearance of the initial lesion to its late depigmented scarring may be several years.

8.1.2. Yaws. The initial lesion of yaws is usually a papule. This painless, pruritic, elevated lesion appears at the site of treponemal invasion after an incubation period of 9–90 days. The site of entry is often a preexisting abrasion, laceration, or insect bite. The treponemes multiply at the infection site, invade subcutaneous lymphatics, cause local lymph node enlargement, and spread systematically through the bloodstream.

The yaws papule enlarges to become a papilloma (frambesioma) that lasts 3–6 months. It may ulcerate and become secondarily infected with bacteria or heal spontaneously, thereby creating a brief period of latency. Secondary yaws lesions then appear in crops on the skin near the initial lesion or elsewhere on the body, including bone and cartilage, as a result of systemic spread and auto-inoculation. Constitutional symptoms are rare, but nocturnal pain of the long bones of the leg due to periostitis and papillomas on the soles of the feet preventing weight bearing are common.

Secondary papillomas are prominent during wet seasons and involute to form micropapules and macules during dry seasons. The lesions heal spontaneously and do not leave permanent scars unless they ulcerate and become secondarily infected by pyogenic bacteria. The disease then enters a noninfectious latent period that may last the lifetime of the patient.

During the first 5 years after infection, the state of latency may be interrupted at any time by the reappearance of infectious yaws lesions. Destructive gummatous lesions appear in the skin or osseous tissue in about 10% of those infected, usually several years after the initial infection. These late lesions mutilate and cripple.

8.1.3. Endemic Syphilis. A primary lesion is rarely seen in endemic syphilis. The first lesions to appear are painless ulcerations on the oropharyngeal mucosa. These are followed by a variety of skin, bone, and joint lesions that are indistinguishable from those of yaws. Skin lesions prefer the moist body surfaces such as the axillary and genital areas. Angular stomatitis or split papules at the corners of the mouth are more common than in yaws and are ideally situated to contaminate drinking vessels.

Osteoperiostitis of the long bones occurs more frequently in endemic syphilis than in yaws. It usually involves the long bones of the lower extremities, causing nocturnal leg pains. Gummas of the nasopharynx, skin, and bone are common in late endemic syphilis and may progress to destructive ulcers. Such ulceration may destroy nasal tissue, producing a terrible disfiguring lesion known as gangosa. Chronic osteoperiostitis and gangosa are still frequently encountered among adult nomadic Bedouins of the Arabian peninsula[29] and occasionally in children and adults suffering from late yaws in West Africa.[22]

8.2. Diagnosis

A presumptive diagnoses of yaws, pinta, or endemic syphilis can usually be made by careful assessment of the clinical features that characterized each infection in appropriate epidemiological settings. Pinta is easily differentiated clinically from yaws, and endemic and venereal syphilis. The differentiation between sporadic cases of yaws and endemic syphilis may not be possible because they may coexist in the same geographic area and their clinical and serological manifestations are indistinguishable.

The most sensitive and specific diagnostic test for treponemes is direct fluorescent antibody (DFA) testing, while the most common confirmatory testing is fluorescent treponemal antibody absorption test (FTA-ABS).[6] Direct fluorescent antibody testing usually requires a central laboratory. Dark-field microscopy, once popular, has decreased as a diagnostic measure due to limited access in endemic areas. With this technique, motile treponemes appear as brilliant coiled threads on a dark background. Lesion exudate can also be collected and fixed on microscope slides and sent to a reference laboratory for microscopic examination using *T. pallidum*-specific monoclonal antibodies.[22] It does not, however, provide differentiating morphologic criteria.[28]

A number of different serological tests are used to detect antibodies produced in response to treponemal infections. These tests were originally designed for use in venereal syphilis and none are specific for a given treponematosis. The most commonly used serological tests are the VDRL and RPR tests, which are highly sensitive in the early stages of disease. They are widely used in screening programs to detect latent yaws or endemic syphilis.

Following adequate penicillin treatment, the titer of VDRL and RPR serological tests declines but, depending upon the duration of the infection, may remain positive for several years or a lifetime. Such is often the case in persons who immigrate from yaws- or endemic syphilis-endemic areas to parts of the world where only venereal syphilis is encountered. Based on reactive serological tests for syphilis, many of these patients are diagnosed and treated inappropriately for active latent venereal syphilis.

9. Control and Prevention

The provision of adequate clothing and soap and water would greatly reduce the transmission of pinta, yaws, and endemic syphilis. Marginal improvements in standards of living together with occasional access to medical care may partly explain why the mutilating complications of yaws and endemic syphilis are seen much less frequently today compared to the preantibiotic era.

The experience gained from pilot programs of the 1950s established a set of principles and procedures that is still valid today and should be used to control yaws and endemic syphilis.[22] The most important principle is to conduct periodic assessments of communities or nomadic groups where one or more members have infectious lesions resulting from recently acquired or relapsed infections. The population of a village or family group should be seen and evaluated at an initial treatment survey and those with active disease should be treated with a single intramuscular injection of 2.4 million units of benzathine penicillin (half-doses for children under 12 years) as should all of their household members and playmates. Control activities should be expanded in such a way that an ever-enlarging disease-free area is established, so that reintroduction of yaws or endemic syphilis by persons from untreated areas is minimized. Because penicillin is inexpensive compared to other medications, many authorities recommend that treatment be given to entire communities that have one or more infectious cases.[14] Treated populations should be reexamined for active yaws within 6 months and at intervals thereafter so that missed cases and their contacts can be treated. The price of freedom from yaws and endemic syphilis is eternal vigilance.

9.1. Antibiotic and Chemotherapeutic Approaches to Prophylaxis

Prophylaxis other than penicillin treatment of contacts of infectious cases of yaws and endemic syphilis is not indicated.

9.2. Immunization

Although theoretically possible to develop, a treponemal vaccine does not exist and the prospects for one are remote.

10. Unresolved Problems

The lack of a cell-free *in vitro* culture system has limited studies of the genetic structure and the immunopathogenesis of the pathogenic treponemes. Control of the endemic treponematoses may result in increased transmission of venereal syphilis, due to loss of partial cross-immunity, with congenital transmission and the serious and potentially fatal cardiovascular and neurological complications. The perennial problem posed by the endemic treponematoses is the low priority given to them by most health authorities when faced with limited resources and more serious disease problems.

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CHAPTER 38

Tetanus

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1. Introduction

Tetanus is a noncommunicable infectious disease of humans and certain animal species, acquired usually through environmental exposure. *Clostridium tetani* is an anaerobic, spore-forming bacilli ubiquitous in the soil. The spores germinate to vegetative bacilli when introduced into the soft tissues of the host under conditions in which the partial pressure of molecular oxygen is low. The vegetative organisms produce a potent neurotoxin that acts on the central nervous system (CNS), leading to the muscular contractions characteristic of the illness. Tetanus can be prevented by active immunization with tetanus toxoid, which is chemically inactivated toxin, or by passive immunization with specific immune globulin as part of postexposure wound prophylaxis. Tetanus toxoid, which protects by inducing production of neutralizing antibodies, is both highly immunogenic and safe. Nearly 100% of recipients of a primary immunization series are protected. Due to increasing use of tetanus toxoid-containing vaccines over decades throughout the world, tetanus is becoming a rare disease; it is relatively more frequent in unimmunized older age groups and in areas where immunization programs are less effective. Although a significant proportion of the global tetanus case burden remains in under-immunized adults and children, a major burden of tetanus in the world is borne by neonates born to mothers who are not adequately immunized and are infected at or soon after birth. However, major progress has been made in the initiative to eliminate neonatal tetanus as a significant public health problem.

2. Historical Background

The clinical characteristics of tetanus are detailed in an ancient Egyptian papyrus and described in the writings of Hippocrates. However, the etiology of tetanus remained unknown until 1884, when two studies were published indicating an infectious process. Carle and Rattone[1] injection drug the contents of a pustule from a fatal human case into the sciatic nerve of rabbits that led to the signs of typical tetanus; the disease could subsequently be passed to other rabbits from infected nervous tissue. Nicolaier[2] induced tetanus in experimental animals after injection of soil samples; he observed gram-positive bacilli in the exudate at the inoculation site but not in nervous tissue, leading him to hypothesize that a locally produced poison led to the neurological symptoms. In 1886, Rosenbach[3] observed spore-forming bacilli in the exudate obtained from a case in a human. In 1889, Kitasato[4] demonstrated that *C. tetani* spores survived heating and germinated under anaerobic conditions; injection of pure cultures reproducibly caused disease in animals. After Behring and Kitasato[5] identified and purified the toxin in 1890, they showed that repeated inoculation of animals with small quantities of toxin led to the production of antibodies in survivors. Preparations of antitoxin from animal sera, particularly horses, became the first means of preventing and treating tetanus. In 1924, Descombey[6] prepared toxoid (chemically altered toxin), which induced neutralizing antibodies without causing illness. Active immunity induced by this preparation opened the way for large-scale tetanus prophylaxis.

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3. Methodology

3.1. Sources of Mortality Data

In the United States and most developed countries, data on tetanus deaths come from national compilations of death certificates (i.e., vital statistics system) listing tetanus as the underlying or contributing cause of death.[7] In such countries, death data probably may be a reasonable, albeit incomplete, estimate of the total deaths from diagnosed tetanus. The National Center for Health Statistics (NCHS) compiles death certificate information from all deaths occurring in the United States.

However, in developing countries where the great majority of deaths occur in the neonatal period primarily outside the hospital, death certificate information may not be available or reliable. In those settings, the completeness of reporting of tetanus deaths, and particularly neonatal tetanus, may be <5%,[8] and only with community-based surveys could accurate estimates of mortality for neonatal tetanus be obtained.[9,10]

3.2. Sources of Morbidity Data

In many developed countries, tetanus is a reportable disease. In the United States, tetanus became notifiable in 1947. The Council of State and Territorial Epidemiologists established in 1990 a uniform set of criteria for disease reporting. The recommended case definition revised in 1996 for a clinically compatible case of tetanus is "Acute onset of hypertonia and/or painful muscular contractions (usually of the muscles of the jaw and neck) and generalized muscle spasms without other apparent medical cause". A confirmed case is a clinically compatible case as reported by a healthcare professional. Case notification is passive, depending on physician initiative, which makes reporting efficiency highly variable. It is likely that only a proportion of the cases actually occurring are reported to national health authorities. In the United States, additional available information on cases reported to the Centers for Disease Control and Prevention (CDC) includes a patient's immunization status, wound history, medical history, postexposure prophylaxis, treatment, and outcome. In the United States during 1972–2005, 614 tetanus deaths were reported to the NCHS compared to 528 deaths reported via the passive surveillance system maintained by the CDC. Also during this period, reported death-to-case ratios (using CDC reports only) have declined from approximately 50 to 10% overall, but ratios vary depending on age and immunization status. Case series from other developed countries have reported death-to-case ratios as low as 10–20%.[11–14] It is likely that there is a bias toward reporting the most severe cases in passive surveillance systems. By matching demographic information for individuals with death following tetanus from both NCHS mortality data and tetanus morbidity reporting, the efficiency of the reporting of tetanus deaths in the United States in the 1980s has been estimated at 40% to CDC and 60% to NCHS.[15] It is likely that substantial under-reporting of cases (and deaths) continues. Similar under-reporting has been estimated for other developed countries.[8]

3.3. Serological Surveys

Serological tests measure the level of circulating antitoxin, which generally correlates with protection from disease when above a threshold of 0.01 International Units (IU)/ml.[16–18] Detectable antitoxin is induced almost exclusively through immunization. Tetanus disease generally does not result in production of circulating antitoxin.[19–21] Therefore, serological surveys, particularly in developed countries, serve an important purpose to measure the impact of immunization in inducing and maintaining population immunity and can supplement the data on the epidemiological characteristics of reported disease.

A national, population-based serosurvey of more than 18,000 people older than 5 years in the United States was conducted during 1988–1994 indicating substantial age and sex differences in antibody prevalence.[22]

Toxin neutralization assays in mice provide results that correlate well with clinical protection since they assess prevention of disease by biologically active antitoxin in a living host, although there is no universally accepted test protocol.[16] Injecton drug the animals with various dilutions of serum and a lethal dose of tetanus toxin allows detection of levels as low as 0.001 IU/ml when compared to a reference serum.[23] However, because the test is time consuming, expensive, and requires animal hosts, a variety of in vitro tests have been employed. These include passive hemagglutination assays (PHA), enzyme-linked immunosorbent assays (ELISA), latex agglutination, and a variety of other methods.[24–27] Sensitivity and specificity vary with the technique. PHA and, more predominantly, ELISA are currently the in vitro techniques used most often to determine tetanus antitoxin levels in population studies. Both assays, are relatively sensitive and specific, although specificity decreases at low antitoxin levels.[24,27] Since PHA measures both IgG and IgM[27] whereas neutralization results from IgG antitoxin,[28] measurement of antitoxin by PHA shortly after the first or second dose of toxoid may report falsely high antibody levels secondary to detection of IgM.

ELISA assays correspond closely with the in vivo neutralization assays at moderate to high levels of antitoxin, but the correlation for standard ELISA is suboptimal at low levels of antitoxin (under 0.2–0.1 IU/ml). Decreased specificity of ELISA at low antitoxin levels can be due to nonspecific antibody binding to contaminant proteins and/or detection of antibody with low avidity to non-neutralizing epitopes. In the United States, for the licensure and regulation of tetanus toxoid-containing products, the US Food and Drug Administration uses 0.1 U/ml when determined by standard ELISA as the surrogate measure of the minimal level of protection. To increase ELISA specificity at low antitoxin levels, modifications such as competitive-antigen or toxin-binding inhibition have been introduced. The comparative value of serological tests for tetanus has been reviewed.[27]

3.4. Laboratory Diagnosis

Tetanus is primarily a clinical diagnosis and the laboratory usually plays little role. Some cases present without a history or evidence of a recent wound. Of cases with wounds, anaerobic culture of wounds is frequently negative, although the yield can be improved by inoculating part of the specimen in cooked-meat medium and heating to 80°C for 5–20 min to destroy nonsporulating competing microorganisms.[29] The organism has been grown from wounds in the absence of clinical symptoms or signs. Serological tests also are of little use, since most tetanus patients do not mount a serological response against the toxin.[30] Although low levels of circulating antitoxin at the time of onset of illness could suggest susceptibility to the agent, detection of antitoxin does not eliminate the diagnosis; several reports have documented antitoxin levels much higher than 0.01 IU/ml (most by in vitro methods, some by neutralization assay) in some patients with classic tetanus at the time of presentation.[27,31] Although such instances are probably rare, tetanus must still be considered in the differential diagnosis as long as the illness is clinically compatible even when circulating antitoxin is greater than 0.01 IU/ml. Detection and quantification of tetanus toxin in serum appear to be diagnostic, although such determination remains a research tool at present.

4. Biological Characteristics of the Organism

C. tetani is a gram-positive, spore-forming, anaerobic bacillus.[29] Flagella attached bilaterally on nonsporulating bacteria add motility. The organism typically measures 0.3–0.5 μm in diameter and 2–2.5 μm in length, although long filamentous forms may be seen in culture. Spores typically form in the terminal position and give the organism its characteristic drumstick-like appearance.

While there may be some tolerance for oxygen when the oxidation–reduction potential is low, for the most part *C. tetani* can be considered an obligate anaerobe. It grows optimally at 33–37°C, although depending on the strain, growth can occur from 14 to 43°C. The organism can be grown on a variety of anaerobic media such as cooked meat, casein hydrolysate, and thioglycolate. Growth is usually characterized by production of gas with a fetid odor. Compact colonies formed in a meshwork of fine filaments are usually seen on blood agar. Addition of reducing substances at neutral to alkaline pH enhances growth.

Formation of spores can be promoted or inhibited by a variety of factors including media composition, temperature, and pH.[22] Sporulation is enhanced in aging cultures and by physiological temperatures (i.e., 37°C), and in the presence of oleic acid, phosphates, 1–2% NaCl, and manganese. In contrast, low pH, extremes of temperature (< 25 or > 41°C), glucose, assorted saturated fatty acids, antibiotics, and potassium can inhibit sporulation.

Spores are highly resistant to environmental agents and may persist in soil for months to years, if not exposed to sunlight.[29] Spores can be destroyed by a variety of disinfectants and heat, although susceptibility varies by agent. Aqueous iodine or 2% glutaraldehyde at pH 7.5–8.5 kills spores within 3 h. In contrast, phenol (5%), formalin (3%), chloramine (1%), and hydrogen hyperoxidates (6%) require 15–24 h to destroy spores. Spores are resistant to boiling, but heating to 120°C for 15–20 min will inactivate them.

C. tetani produces two exotoxins: tetanolysin and tetanospasmin.[29] Tetanolysin is an oxygen-sensitive hemolysin related to streptolysin and the theta toxin of *C. perfrigens* that may play a limited role in establishing infection.[29] Tetanospasmin, the cause of the clinical signs and symptoms of tetanus, is a highly potent neurotoxin that accumulates intracellularly during the logarithmic growth phase and is released into the medium upon autolysis. Toxin production is under the control of a plasmid.[32] The molecule of approximately 150,000 Da is synthesized as a single polypeptide chain that is cleaved into light and heavy chains bound by a disulfide bond when released into the medium.[33]

The toxin is one of the most potent poisons on a weight basis, although sensitivity varies considerably by species. Guinea pigs are particularly sensitive, with doses of 0.3 ng/kg usually fatal. About 1 ng/kg will kill a mouse, while the estimated minimum human lethal dose is < 2.5 ng/kg. Monkeys, sheep, goats, and horses also are extremely susceptible to the effects of the toxin, while cats, dogs, and particularly birds and poikilotherms are relatively

resistant.[34] The differences in species susceptibility cannot be explained by differences in circulating antitoxin but may be due to differences in toxin binding to peripheral motor neuron terminals.[29,35]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

In spite of the availability of a highly effective immunizing agent for decades, tetanus continues to exert a substantial health impact throughout the world. It is clear however, that while the number of cases of tetanus in neonates (tetanus during the first month of life), children and adults are decreasing, the past burden of neonatal tetanus had been greatly underestimated. Given its substantial impact in developing countries, the control of neonatal and nonneonatal tetanus is the subject of a major effort of Expanded Programme on Immunization (EPI) by routine immunization in infancy with diphtheria–tetanus–pertussis (DTP) and by immunization of women of reproductive age with tetanus toxoid.

In the 1960s, the incidence of tetanus neonatorum in developing nations had been estimated to be under 50,000 cases annually, or approximately 0.5/1,000 live births overall.[31] By 1973, recognizing problems in surveillance, the estimate of annual tetanus deaths worldwide shifted upward to 1 million, with 60–90% of that mortality ascribed to tetanus neonatorum.[36] However, in the 1980s, using more accurate information collected in community-based surveys of infant deaths, despite further advances in immunization of pregnant women, an estimated 1.2 million deaths due to neonatal tetanus (about 10 deaths/1,000 live births) and an estimated 30,000 deaths due to maternal tetanus were occurring each year.[9,37] At the same time, there were over 600,000 estimated cases of nonneonatal tetanus associated with an estimated 120,000–300,000 deaths.[38] Ongoing efforts in the prevention and elimination of neonatal tetanus have led to a decline in the estimated number of neonatal tetanus deaths per year; by 1988, there were 787,000 annual deaths (6 deaths/1,000 live births).[39] In 1989, the World Health Assembly of the World Health Organization (WHO) adopted a resolution to eliminate neonatal tetanus worldwide by the year 1995, and this was reaffirmed in 1990 at the World Summit for Children. The objective of eliminating neonatal tetanus—defined as less than one case per 1,000 live births for each health district—was not met by the target year, when an estimated 300,000 deaths due to neonatal tetanus occurred. Advances toward the goal were due in large part to the use of the "high-risk" approach toward prevention of neonatal tetanus, in which supplemental immunization campaigns for all women of childbearing age are held in selected high-risk areas identified by surveillance or other indicators. The impact of increasing immunization coverage of pregnant women with tetanus toxoid (from 9% in 1980 to 69% in 2006),[40] the success of targeted mass immunization in high-risk populations and the increasing prevalence of clean deliveries are currently estimated to prevent 1.3 million deaths each year due to neonatal tetanus. Despite this marked progress in prevention, approximately 180,000 neonatal tetanus deaths continue to occur each year as of 2002 (1.4 deaths/1,000 live births).[41] Continued improvements in vaccination coverage, hygiene and childbirth practices, and surveillance to identify remaining high-risk areas and target them for intensified intervention efforts, as well as continuing the strengthening of routine childhood immunization services, will contribute in further decreasing the global burden of maternal and neonatal tetanus.

In most developed countries, improved hygiene and childbirth practices (associated with an increasing proportion of births delivered by trained health professionals in hospitals), improvements in wound care, reduction in exposure to tetanus spores, and active immunization have led to major declines in tetanus incidence since the 1950s. In the United States, death certificate data from 1920 onward indicate a decline in annual tetanus death rates, which may have accelerated with the use of equine antitoxin in prophylaxis and treatment beginning in the mid-1920s (Figure 1).[42] Cases of tetanus have been monitored nationally since 1947, when the incidence was 0.39/100,000 total population; secular trends in tetanus occurrence since that time reflect changes in wound management and the use of toxoid. A continual decline in reported cases occurred until 1976 to about 0.04/100,000. Since then, a less pronounced decline in incidence rates has continued to the current 0.010/100,000.[43–50]

As indicated by the trends in overall death rates, the occurrence of neonatal tetanus in the United States was already on the decline prior to widespread tetanus toxoid use in women because of an increasing number of births in hospitals and improvements in postpartum hygiene. Deaths due to tetanus in those less than 1 year old, generally reflecting neonatal disease, declined from 0.64/1,000 live births in 1900 to 0.07 in 1930 to 0.01 by the 1960s. Using age-specific case reporting beginning in 1961, tetanus incidence could be directly monitored in those less than 1 year old and showed the same downward trend. From 1967 to 1968, the incidence rates of reported cases and of reported tetanus deaths in those less than 1 year old declined abruptly. This abrupt decline occurred primarily because of a decrease of reported cases in Texas following a dedicated effort to reach women at risk.[51]

Of the 32 cases reported in the first 4 weeks of life in the United States during 1972–2005, 29 were in infants

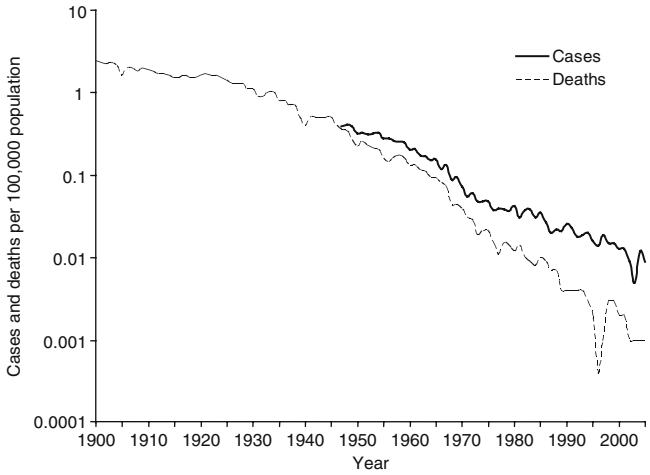


Figure 1. Reported mortality and case incidence rates of tetanus in the United States, 1900 to 2005. Not all states reported tetanus-associated deaths until after 1932; the estimated rates shown here are based on the population of the reporting states. National reporting of cases began in 1947. (Source: Centers for Disease Control and Prevention.)

born outside of a hospital.[49–50] Nineteen of the cases (59%) occurred in Texas, which had only 9% of US births. Only five mothers had a history of ever having received tetanus toxoid, and only one was known to have received more than one dose. Twenty of the 29 case-infants with known outcome survived. Only three cases of neonatal tetanus have been reported in the United States after 1989; one was to an Hispanic mother.

5.2. Epidemic Behavior

C. tetani spreads from the environment to humans generally through contamination of wounds. The organism cannot spread from person to person; tetanus is a non-contagious infectious disease. Nevertheless, outbreaks of tetanus in drug users (particularly among heroin addicts) have been reported.[52,53] In the United States during the 1990s, an increase of tetanus cases was reported among illegal injecton drug users, a recognized risk group, which halted a decline in the average annual incidence rates among persons 20–39 years of age.[46–48]

5.3. Geographic Distribution

C. tetani organisms are distributed across the globe. However, the greatest concentrations are in warm climates with moist, fertile soil.[54] The highest rates of tetanus disease have historically occurred in the developing world, particularly in countries near the equator.[36,54] Since the introduction and wide use of tetanus toxoid, geographic distributions reflect areas with under-immunized populations. In the United States, tetanus had been predominantly a disease of the Southeast; however, all regions currently report cases with approximately the same incidence. The occurrence of tetanus in regions at high altitude may be lower than in low-lying areas, although the reasons for this phenomenon are not understood.[55]

5.4. Temporal Distribution

In the pre-toxoid era, tetanus was generally distinctly seasonal, with a mid-summer or "rainy" season peak in temperate areas. This is consistent with multiplication of the organisms in the soil and sporulation but more likely also reflects more frequent host behaviors associated with injury during the warmer months.[44,54]

5.5. Age

As noted earlier, tetanus in developing countries occurs most frequently in neonates. Even in the United States in the 1950s, over one-third of tetanus deaths were in children under 1 year of age.[43] Aside from neonatal tetanus, the substantial proportion of victims in developing nations are older children and young adults. Wherever immunization programs are in place, tetanus occurrence declines.[54] In the face of immunization programs, age (and sex) distributions shift to reflect the under-immunized. In the United States since 1965, average annual age-specific tetanus incidence rates reflect declines in all age groups. However, less dramatic declines were registered in the elderly, while drastic declines were reported for younger age groups. Thus, in

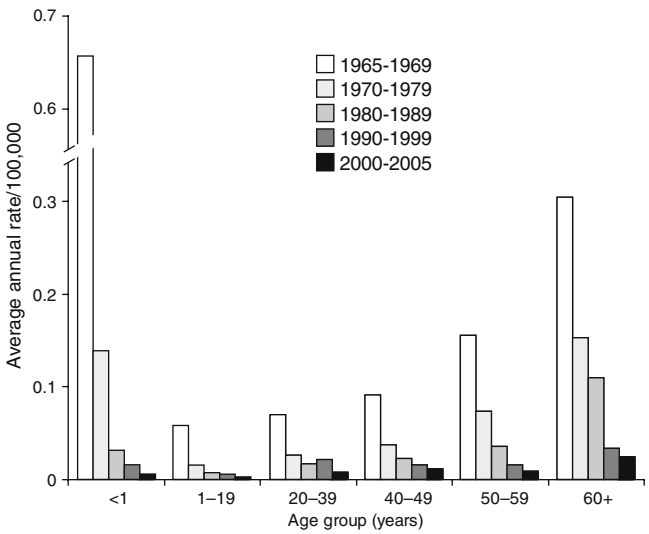


Figure 2. Age group-specific incidence rates of reported tetanus in the United States, 1965 to 2005. (Source: Centers for Disease Control and Prevention.)

recent years, the elderly have become the group at highest risk for tetanus (Figure 2). Among 142 cases reported in the United States in 2001–2005, the average annual incidence was 0.003 per 100,000 among persons less than 20 years of age compared with 0.024 per 100,000 among persons 60 years of age and older, an eightfold greater risk of acquiring tetanus.[50] A population-based serosurvey of immunity to tetanus conducted in the United States during 1988–1994 revealed that 70% of the population aged 6 years and above had tetanus antibody levels defined as protective. Nevertheless, the prevalence of tetanus antibody levels defined as protective declined with age, from >90% in children aged 6–11 years to 31% in people aged 70 years and older.[22]

5.6. Sex

In much of the world, reported tetanus appears to be more common in males than in females at all ages.[8,54] Although there may be a biologic reason, the general male predominance of neonatal tetanus in developing countries may in part be due to the possibility that parents of males may seek medical attention more frequently than do parents of females, or may apply substances to the umbilical stump associated with a higher risk of tetanus.[36] Among children and adults, males generally appear to have a somewhat higher reported incidence, which could perhaps be due to exposure factors, such as injury-incurring behaviors outdoors, and/or surveillance artifact.

In the United States for the period 1972–2005, two-thirds of the 32 neonatal tetanus cases were in males.[49,50] Outside of the neonatal period, the overall pattern of disease by sex seen in the United States is different from that seen in developing countries because males entering military service during World War II (and subsequently) were routinely vaccinated. During 2001–2005, among persons aged 1 month to 69 years, the incidence of reported tetanus was higher in males than females (0.011 vs. 0.005 per 100,000, respectively). Among persons aged 70 years and older (i.e., in a cohort born prior to routine infant and childhood immunization but in which the majority of males served in the military), the incidence was lower in males than females (0.015 vs. 0.030 per 100,000, respectively).[49,50]

5.7. Race

In the 1950s in the United States, nonwhite individuals had an incidence more than five times that in whites, and rates of neonatal tetanus were ten times higher.[43] In the United States in 1972–1989, the estimated average annual incidence rate was 0.02/100,000 for whites, 0.05/100,000 for blacks, and 0.12/100,000 for all other races.[49] These differences are felt to be attributable primarily to lower levels of tetanus toxoid immunization in the nonwhite populations, but also could reflect differences in health care following injury. For tetanus cases reported in the United States from 2001 to 2005, the incidence no longer differed between white and black populations.[50] However, adult, non-US-born immigrants, particularly Hispanics, may be at substantial increased risk for tetanus compared with the rest of the population, which may be attributable to a combination of injection drug use and underimmunization. From 1992 to 2001, Hispanics had an average annual incidence of tetanus that was 2.2 times higher than that of non-Hispanics; 38% of cases in Hispanics were among injection drug users. During these years, Hispanics made up 15% of tetanus cases that were not in injection drug users but 66% of cases among injection drug users.[49]

5.8. Occupation and Location

Among cases reported in the United States during 1972–2001, the relatively larger proportion of tetanus-associated wounds occurred in outdoor settings—in yard, farm, and gardening settings or following major trauma outdoors. During 1972–2001, however, case reports indicated that specific settings where tetanus-associated wounds were incurred in decreasing frequency indoors, in farm, gardening, hospital, and then factory settings.[45,49]

5.9. Occurrence in Different Settings

Because of wound and field conditions, war injuries frequently were associated with tetanus; it has been estimated that, prior to the availability of toxoid, 1 out of 100 serious wounds in battle conditions was complicated by tetanus. As a result of routine tetanus toxoid prophylaxis and vigorous prophylaxis in wound management, only 12 cases of tetanus occurred among 2.73 million wounded US Army personnel on all fronts in World War II (0.44/100,000) versus 70 out of 520,000 wounded in World War I (13.4/100,000).[56]

Parenteral drug abuse, both intravenous and subcutaneous, is known to place individuals at higher risk compared to the general population. Drug users may be at higher risk for tetanus because of nonhygienic injection practices; however, heroin is frequently mixed with quinine, which lowers the redox potential at the injection site, favors growth of anaerobes, and may create an ideal environment for *C. tetani* to germinate.[57] Patients with a reported history of illicit injection drug use accounted for 5% of tetanus cases overall from 1972 to 2001; drug use-associated cases increased during the 1990s to reach 12% of reported cases during 1992–2001.[49] Black tar heroin was reported as the common

injected drug for 71% of 14 heroin users among cases in during 1998–2000.[48]

Operative procedures, particularly bowel surgery, infrequently can put some individuals at risk, as can dental procedures, puerperal sepsis, and septic abortion.[37,58] Body piercings have been reported as a procedure associated with tetanus.[59,60]

5.10. Socioeconomic Factors

The incidence and complications of tetanus are inversely related to access to (1) routine prophylactic immunization, (2) immunization with appropriate wound care following wounds, and (3) care following onset of symptoms. Availability and use of these services probably correlate directly with socioeconomic status. Data collection on reported cases has not included indicators of socioeconomic status. Case-fatality ratios during 1972–1989 differed by race—31% for whites, 43% for blacks, and 34% for all other races—which may reflect differences both in access to health care and in immune status; in this case series, having two or more prior doses of tetanus toxoid put one at lower risk of death from tetanus.[49]

The differences in racial incidence in earlier series and the characteristics of mothers of patients with neonatal tetanus are strongly felt to reflect poorer immunization levels associated with lower socioeconomic status. The population-based serological survey of tetanus immunity in the United States also suggested that seroprevalence was positively correlated with education: the higher the educational level, the higher the tetanus seroprevalence rates.[22] These data suggest that compliance with routine vaccination recommendations (and access to postexposure wound prophylaxis) may also be correlated with higher educational levels.

6. Mechanisms and Routes of Transmission

6.1. Children and Adults

Outside the neonatal period, a variety of conditions have been associated with tetanus. Tetanus is caused by the in vivo elaboration of tetanospasmin. Spores of *C. tetani* are introduced through breaks in the skin epithelium; germination of the spores with production of toxin leads to disease. In the United States, information is available for 1,805 nonneonatal cases reported from 1972 through 2001.[49] Acute injuries were the predisposing event in 1,372 (76%), while chronic wounds or other medical conditions were associated with 289 cases (16%). Parenteral drug abuse was the only predisposing condition in 5% and was present in an additional 1%. For 8% of patients, no acute injury, chronic wound, or associated medical condition was known. Of the patients reporting acute injuries, puncture wounds were most common (38%) followed by lacerations (31%). The circumstances of the wound were known for 78% of these cases. Injuries in outdoor settings (e.g., yard, garden, or farm settings) accounted for 29% of cases with acute wounds, injuries incurred indoors accounted for 21% of cases, and 10% were incurred under special circumstances. These included animal bites and/or scratches in 3%, major trauma (burns, bullet wounds, and compound fractures) in 4%, and surgical wounds, many of which were contaminated (e.g., bowel wounds), in 3%.

Chronic medical conditions associated with tetanus included decubitus ulcers, gangrene, and frostbite, as well as dental and other abscesses and parenteral drug abuse.[45–49,52–54] Cases without known wounds or portals of entry have been described for many years.[54] In some instances, these are thought to arise from minor wounds not recalled by those patients.[61] Estimated incidence among persons with diabetes mellitus is higher than the general population by approximately five-fold.[49]

Other conditions associated with tetanus include foreign bodies and contaminated injections, e.g., illicit injected drug use, body piercing, and the practice of tattooing.[52,53,59,60]

6.2. Neonates

Neonatal tetanus occurs in newborn infants born to unimmunized women following infection by *C. tetani* (most commonly at the umbilical cord stump). Cases are associated with overtly unsterile surfaces used in childbirth, delivery personnel untrained in sterile care of the umbilical cord and stump, and particularly with births followed by unhygienic cultural rituals involving the umbilical stump, such as application of herbs, clarified butter, or animal dung.[8,10,51,62] The risk of disease is lowered by avoiding the application of these substances, as well as "clean" delivery practices[63–65] and potentially antibiotic ointment application.[66,67] Training of birth attendants in clean delivery practices (use of soap and water to clean hands, use of clean surfaces and a sterile cutting device) is a major step in not only preventing neonatal tetanus but also reducing other causes of neonatal mortality.[63–65]

Neonatal tetanus can be prevented through transplacental passage of circulating antitoxin from immunized mother to infant, most of which is in the IgG1 subclass.[16,17,68] Although decreasingly, many newborns in the world continue to be susceptible because their mothers have not received two or more doses of tetanus toxoid; it was estimated that even as of 2006, only 69% of pregnant women in developing countries have received at least two recent doses of tetanus toxoid.[40]

7. Pathogenesis and Immunity

7.1. Pathogenesis

Tetanus results when spores inoculated through the epithelium germinate, leading to the production of tetanospasmin and its transport to the CNS. The period of time between an identified acute injury and onset of tetanus signs and symptoms is 3 days to 3 weeks for the majority of cases. In the United States in 1972–2001, the median incubation period in reported cases was 7 days.[49]

Germination is enhanced by anaerobic conditions associated with significant tissue injury and accumulation of lactic acid.[29] Calcium, as present in soil, may increase the likelihood that injection drug injection drug spores germinate because it can increase local necrosis.[69] Toxin injection drug or produced in the subcutaneous tissue appears to enter muscle first; tetanus can be prevented in experimental animal models by treating the muscle with antitoxin prior to subcutaneous administration of toxin.[70]

Once in the muscle, toxin can reach the bloodstream via the lymphatics. Toxin is disseminated hematogenously: studies show that when toxin is injection drug subcutaneously or intramuscularly, it reaches the bloodstream and from there is distributed to tissues. However, the toxin does not cross the blood–brain barrier; toxin gains access to the CNS by neuronal transport.[70,72–74] Toxin gains entry to the nerve by binding to lipid raft microdomains containing specific polysiagangliosides and glycoproteins at the motor end plates of muscle nerves[75] and is transported to the ventral horns of the spinal cord or motor nuclei of cranial nerves. Transynaptic transport of intact toxin allows transfer to inhibitory neurons in the spinal cord and wider dissemination within the CNS. Different portions of the heavy chain of tetanospasmin have properties necessary for the separate steps of neuron receptor binding, internalization, intraaxonal transport, and membrane translocation. Once released at its site of action, the freed light chain is an endopeptidase that cleaves a synaptic vesicle membrane protein necessary for neurotransmitter release.[35,74,76]

By interfering with the release of the inhibitory neurotransmitters glycine and γ-aminobutyric acid from presynaptic inhibitory fibers, the light chain of tetanospasmin prevents inhibition of spinal cord reflex arcs. This allows excitatory reflexes to multiply unchecked and results in the classic spasms of tetanus.[70] In addition, the toxin light chain can impede release of a variety of other neurotransmitters including acetylcholine when binding to and affecting autonomic and somatic nerves.[76] Action at these sites accounts for autonomic dysfunction in cases of severe cases of tetanus and the flaccid palsy of cranial nerves in cephalic tetanus.

7.2. Immunity

Immunity to tetanus does not generally follow disease, since the quantity of toxin that causes illness is usually less than the quantity needed to induce an immune response.[30] Second cases of tetanus have been reported.[19–21,77] Most serosurveys in unimmunized populations fail to find evidence for natural immunity. A few authors reported serosurveys in unimmunized populations, particularly of developing countries, in which some persons had antibodies.[78,79] However, the assays generally used are now known to be nonspecific at low antitoxin levels; additionally, some subjects may have received prior immunizations. Asymptomatic infection/colonization, presumably of the intestine, may very rarely result in the production of antitoxin.[61]

It is generally accepted that a circulating level of antitoxin of 0.01 IU/ml by neutralization assay is the minimum level needed to assure protection against tetanus.[16–18] This comes from experiments both with humans and with animals. Guinea pigs with serum antitoxin levels of 0.01 antitoxin unit (AU)/ml (prior to international standardization of the assay) or higher following active or passive immunization survived challenges with fixed lethal doses of *C. tetani* spores.[80] Looney reviewed animal studies of active immunization that suggested incremental protection at 0.01–0.1 AU/ml.[81] A review by McComb[18] in 1964 reported that clinical tetanus could be prevented in horses by administration of 1,500 IU/ml of antitoxin after acute injury, which corresponded to a serum level of 0.01 IU/ml. Following immunization with tetanus toxoid, Wolters and Dehmel[82] were able to resist challenge in themselves with "2–3 fatal doses" of tetanus spores. They had achieved serum levels of 0.007–0.01 AU/ml. Further evidence that 0.01 IU/ml was protective came from trials to prevent neonatal tetanus. Induction of antitoxin levels of 0.01 IU/ml or higher in a sample of mothers was correlated with protection of infants from neonatal tetanus.[16]

The appropriate minimum protective level of circulating tetanus antitoxin has been challenged by reports of adult and neonatal tetanus patients who had serum levels greater than 0.01 IU/ml at the time they presented with clinical tetanus.[27,31,83] The highest level measured by neutralization assay noted was 0.16 IU/ml. Large-scale production of toxin from heavily necrotic wounds or uptake of toxin through nerve endings in tissue with inadequate circulation may in some instances prevent neutralization of toxin at antitoxin levels of 0.01 IU/ml or above. Whatever the explanation, these data suggest that the 0.01 IU/ml minimum level of protection by neutralization assay cannot be considered absolute.

8. Patterns of Host Response

8.1. Clinical Features in Children and Adults

The reported incubation period for tetanus ranges from 1 day to several months. However, the majority of cases occur within 3 days to 3 weeks after an injury. In the United States, the median incubation period for cases with injury-associated wounds reported between 1972 and 2001 was 7 days and the range was 0–178 days.[49] The incubation period can vary with distance between the wound and the CNS. The longest periods are reported for injuries farthest away from the CNS, while injuries to the head and trunk have the shortest incubation periods.[70,84] Historically, shorter incubation periods have been associated with more severe disease and higher case-fatality,[11,44,85] although this has not been the case in patients recently reported in the United States.[49]

Clinical tetanus can take one of three forms: (1) localized, (2) generalized, and (3) cephalic.[84] Local tetanus, which is uncommon in humans, consists of painful spasms in a confined area around the site of the original injury.[86] The spasms may persist for weeks to months. The prognosis of tetanus that remains localized is excellent; however, localized tetanus can progress to generalized tetanus, which carries a more ominous prognosis. Over 80% of cases of tetanus fit the generalized tetanus category. Patients most often present with trismus (lockjaw), i.e., spasm of the muscles of mastication. Risus sardonicus, the characteristic facial expression, occurs with trismus and spasm of the facial muscles. It consists of raised eyebrows, tight closure of the eyelids, wrinkling of the forehead, and extension of the corners of the mouth laterally. Initial signs may be followed by involvement of multiple other muscle groups, hyperreflexia, and generalized hypertonicity. Tetanospasms, generalized tetanic seizure activity, consist of sudden painful contraction of all muscle groups, resulting in opisthotonos, adduction and flexion at the shoulders, flexion of the elbows and wrists, and extension of the legs. Spasm of the glottis can cause immediate death. Autonomic nervous system abnormalities consisting of arterial hypertension or hypotension, tachycardia, and arrhythmias, among other manifestations are frequently reported in severe cases.[87] Complications can result directly from the actions of the toxin, from spasms, or from chronic debility. Reported death-to-case ratios for generalized tetanus with medical care have varied from 10 to 75%. With good intensive care, mortality should be less than 20%. The greatest mortality occurs at the extremes of age.[10–14,49]

Cephalic tetanus occurs with injuries or lesions of the head and face.[84] It is characterized by short incubation periods, 1–2 days, and by atonic cranial nerve palsies particularly involving nerves III, IV, VII, IX, X, and XII, singly or in combination. Trismus may be present. Cephalic tetanus may progress to generalized tetanus. Of 563 tetanus cases reported in the United States in 1972–2001 with data on clinical presentation, 88% were generalized, 12% were localized, and $<1\%$ were cephalic.[49]

8.2. Clinical Features in Neonatal Tetanus

Tetanus occurring within the first month of life is a form of generalized tetanus. Symptoms of neonatal tetanus generally begin 3–14 days after birth but can occur from 1 to 28 days of age. The illness starts with poor sucking and excessive crying. This is followed by variable degrees of trismus, difficulty swallowing, opisthotonos, and other tetanic spasms. The case-fatality ratio of neonatal tetanus is 10–90% with therapy, depending on the intensity of supportive care.[6,10,11,14] Overall, the EPI of the WHO estimates that 80% of affected infants die in low-income countries.[8]

8.3. Diagnosis

The diagnosis of tetanus is made primarily on clinical grounds. Generalized stiffness, hyperreflexia, trismus, and the characteristic generalized tetanic spasms strongly support the diagnosis. Absence of changes in the state of consciousness or sensory involvement add further support. The epidemiological history can also be helpful. A preceding history of a wound, particularly if contaminated by soil, is suggestive, although not all cases will have such histories. Lack of a history/record of adequate immunization or wound care is typical. Given the high efficacy of a course of tetanus toxoid, a patient with a clinically compatible case who presents with a history of complete primary series of toxoid and maintenance boosters should undergo an exhaustive workup for other potential causes. The diagnosis of tetanus can be reached if this workup fails to identify another cause. Significant rises in circulating antitoxin are not reliably seen in tetanus and cannot be expected to aid in the diagnosis.[30] Testing for antitoxin using ELISA can be performed on a rapid basis to assist in assessment of immune status; absence of circulating antitoxin at 0.10 IU/ml or greater (by classic ELISA) when the patient is first seen supports the diagnosis. In the event of isolation of the organism from a wound, this is strongly supportive.

The differential diagnosis will depend on the clinical signs and symptoms.[84,88] Trismus may be caused by dental caries, tonsillitis, peritonsillar abscesses, parotitis, temporomandibular joint dysfunction, and CNS disturbances other than tetanus. Hyperreflexia may be seen in rabies; however,

rabies is more often associated with changes in the state of consciousness, and the seizures of rabies are more often clonic in contrast to the tonic seizures of tetanus. The history of an animal bite is generally present with rabies but could be present in tetanus. Encephalitis also can result in spasms, but, like rabies, is much more likely to be associated with changes in the state of consciousness. In addition to infections, a variety of poisonings and metabolic conditions can simulate tetanus. Hypocalcemic tetany can cause generalized muscle spasms but is not usually associated with trismus.[89] Strychnine poisoning, which also causes disinhibition of spinal cord reflex arcs, can resemble generalized tetanus clinically.[90] However, strychnine poisoning is usually not associated with muscle rigidity between spasms and is rarely associated with temperature elevations, which are frequent in severe tetanus. Dystonias caused by phenothiazines also should be considered in the differential diagnosis. Finally, hysteria can mimic tetanus, although such patients tend to relax on prolonged observation and their seizure-like activity tends to be clonic rather than tonic.

9. Control and Prevention

Tetanus is an entirely preventable disease. Prevention begins with universal, routine immunization and with appropriate attention toward averting wounds and injuries. A routine immunization program should ensure that the entire population has received a primary series (at least three doses) of tetanus toxoid with booster doses as needed. Substantial health benefits are derived from combined immunization with diphtheria and tetanus toxoids and pertussis vaccine (DTP) in childhood or (Tdap) in adulthood (one-time dose) or diphtheria toxoid (Td) otherwise in adulthood. Vaccination coverage is high in the United States, with 92% of children 19–35 months of age having received four or more doses of a tetanus toxoid-containing vaccine[91] and 96% of children entering school.[92] It has been estimated that since 1990, more than 70% of target children in the world had received three doses of DTP in the first year of life annually; current estimates are approximately 80%.[40]

Once injuries occur, efforts should be made to prevent contamination with spores and to mitigate those conditions that predispose to germination of spores. Wounds should be carefully cleaned and surgically debrided when necessary to remove devitalized tissue, which can serve as a nidus for infection, followed by tetanus toxoid (and tetanus immune globulin, when needed).[93,94] Since neonatal tetanus is the major worldwide cause of tetanus morbidity and mortality, continued attention must be paid to covering umbilical cord stumps with sterile or uncontaminated dressings. Training of midwives in proper sterile techniques has been demonstrated to reduce the incidence of neonatal tetanus substantially (although not as effectively as immunization of the mother) as well as result in declines in neonatal mortality due to causes other than tetanus.[63–65]

Immunization histories for cases in the United States in 1998–2000 were verified; the immunization status was known for 38% (49) of the 129 nonneonatal tetanus cases reported.[48] Twenty (15%) of the 129 cases had received at least a primary series (i.e., three or more doses) of tetanus toxoid before onset of tetanus; only eight reported that the last dose of the primary series or booster dose of toxoid was received less than 10 years before the illness. Of the 90 patients in 1998–2000 with tetanus following acute wounds and available medical history, 33 (37%) obtained medical care for the injury; a tetanus toxoid-containing vaccine was given as prophylaxis in wound management to 20 (61%) of these and TIG was given to only 3 although the vast majority were apparently eligible. Thus, virtually all cases of tetanus could be prevented with routine immunization and appropriate wound management.

9.1. Passive Immunization

Although animal models suggest that prompt passive immunization is protective against the development of tetanus following injury, there is limited information in humans; appropriately controlled trials have not been performed. Passive immunization with equine antitoxin for treatment and for prophylaxis following wounds became common practice in World War I, with a decrease in wound-associated tetanus at the same time that other improvements in wound care were becoming common practice.[85] Passive immunity conferred by equine antitoxin is of limited duration. The half-life of refined equine antitoxin in humans is less than 2 weeks and may be shorter in some individuals.[95] A major disadvantage is the risk of immediate hypersensitivity reactions or serum sickness.[96]

Tetanus immune globulin (TIG) is a human antibody preparation prepared by cold-ethanol fractionation of the plasma of hyperimmunized adults. TIG was introduced in the early 1960s and was found to have a fairly constant half-life of 28 days in humans.[18] Allergic and serum sickness reactions to equine antitoxin, along with its shorter half-life, made TIG more attractive for passive immunization. TIG has been shown not to pose a risk of hepatitis (B or C) transmission. TIG and other purified immune globulins pose no risk of human immunodeficiency virus (HIV) transmission, since donors are screened to be at low risk, serum is screened for antibody to HIV prior to serum pooling, and, most important, cold-ethanol fractionation inactivates HIV.[97] As an added

safeguard against the possible transmission of hepatitis C virus, the Food and Drug Administration (FDA) is working with the manufacturers to include an additional inactivation/removal step. It is distributed at a concentration of 250 IU/ml in 1-ml vials. Even in the developing world, TIG is beginning to supplant equine antitoxin as it becomes more widely available. Tetanus toxoid is always given when TIG is given to induce persistence of immunity beyond 28 days in those who received a dose or more of toxoid in the past and to initiate active immunization in those without any prior exposure.[98] In contrast to equine antitoxin, the currently licensed TIG products in the United States should never be administered intravenously, because of aggregates. It is intended solely for intramuscular use.

9.2. Active Immunization

Tetanus toxoid is one of the best immunogens available today. A primary series will induce protective levels of circulating antitoxin in virtually 100% of recipients.[16,99] Protective levels usually last at least 10 years and in the majority will be present even up to 25 years after the last dose.[100]

9.2.1. Production and Testing of Tetanus Toxoid. To produce toxoid, *C. tetani* is cultured in large-capacity fermentors in liquid medium free of allergenic substances. The culture filtrate is precipitated with methanol or ammonium sulfate to purify partially extracellular toxin. Toxin is further purified and detoxified with 40% formaldehyde at 37°C. By WHO standards, the final product should contain 0.5% formaldehyde or less. In the United States, minimum requirements stipulate a residual formaldehyde content of 0.02% or less. A single dose of adsorbed toxoid must contain less than 1.25 mg of aluminum. Tetanus and Diphtheria Toxoids and Acellular Pertussis Vaccine, Adsorbed (For Adult Use) (Tdap) products used in the United States, and some preparations of Tetanus and Diphtheria Toxoids, Adsorbed (For Adult Use) (Td), are either thimerosal free or contain only trace amounts ($< 0.00012\%$) from the manufacturing process. Multiple-dose packaging includes 2-phenoxyethanol as preservative, which is present in some single-dose preparations. Some Td products and adsorbed tetanus toxoid multiple-dose preparations contain 0.0033–0.01% thimerosal as preservative (as does the licensed but generally unavailable fluid toxoid).

Potency is determined by animal bioassays: for the fluid preparation, immunized guinea pigs are tested for survival after a toxin challenge; for precipitated toxoid, a serum pool from immunized guinea pigs must exceed 2 IU/ml of tetanus antitoxin. The toxoid content of commercial products is assessed by flocculation; this measure of toxoid protein content (Lf) does not equate directly with immunogenicity as measured by potency in guinea pigs. Adsorbed products available in the United States have a content of 4–10 Lf per dose; fluid products contain 4–5 Lf.

Tetanus toxoid is available in several preparations: Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed (whole-cell DTP; no longer used in the United States or most industrialized countries; also referred to as DPT outside the United States), Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine, Adsorbed (DTaP), and Diphtheria and Tetanus Toxoids Adsorbed (For Pediatric Use) (DT) used in infants and children under 7 years of age. Universal use of DTaP (or DTP where relevant) in infancy and childhood is recommended unless there are contraindications to pertussis vaccine. DTaP is also licensed with other antigens: Haemophilus influenzae type b (Hib) conjugate vaccine, hepatitis B (Hep B), and/or inactivated poliovirus vaccine (IPV) (available as DTaP-Hib, DTaP-Hep B-IPV, and DTaP-Hib-IPV). Td is for use in persons 7 years of age and older; it contains less diphtheria toxoid (2 Lf or less) than the pediatric preparation (over 10 Lf). Td is the preferred preparation for tetanus prophylaxis in adults under all circumstances because the vast majority of adults in need of tetanus toxoid are likely to be susceptible to diphtheria. In the United States, adsorbed (solitary) tetanus toxoid and fluid tetanus toxoid are produced by a single manufacturer for use in persons 7 years of age and older; the latter is only available in limited quantities. In 2005, two Tdap products (reduced content of pertussis antigens and reduced diphtheria toxoid as compared to the pediatric preparation [DTaP]) were licensed for one-time use in persons 10–18 years of age (BOOSTRIX®, GlaxoSmithKline Biologicals, Rixensart, Belgium) and 11–64 years of age (ADACEL®, sanofi pasteur, Inc., Swiftwater, Pennsylvania), depending on the respective product, for protection against tetanus, diphtheria and pertussis; these products can be used in wound prophylaxis if indicated.

In the United States, aluminum hydroxide or aluminum phosphate is used as an adjuvant. These salts allow an adequate immune response after fewer doses of toxoid than with the fluid preparation.[101] Since fluid and adsorbed preparations do not differ substantially with regard to adverse events, adsorbed toxoid is preferred because it confers protective levels of antitoxin for a longer period of time.[101,102] Response to either form of toxoid as a booster dose is equally brisk. In combined active–passive immunization, TIG does not substantially alter the response to adsorbed toxoid as it does with fluid toxoid.[98,103] In some countries other than the United States, a calcium phosphate adsorbed product is also available. The adsorbed toxoid is administered intramuscularly; fluid preparations can be given subcutaneously. Either can be given by jet-injector.

Table 1. Recommendations for Primary Immunization and Booster Doses with Tetanus Toxoid by Age at Beginning Immunization, United States, 2008*

	Age group			
	<1 year	1–6 years		≥7 years
Vaccine	DTaP* or DT+	DTaP*	DT+	Td/Tdap‖
Interval before				
Dose 1	First visit++	First visit	First visit	First visit
Dose 2	1–2 months	1–2 months	1–2 months	1–2 months
Dose 3	1–2 months	1–2 months	6–12 months	6–12 months
Dose 4	approximately 1 year	approximately 1 year	–	–
Boosters	–§	–§	–¶	–‖

* DTaP is the pertussis vaccine preparation for all doses in the series in the United States. Whole-cell DTP is an acceptable alternative throughout the world. Combination DTaP preparations available in the United States include DTaP-HepB-IPV for doses 1–3 and DTaP-Hib for dose four in the series only. A DTaP-Hib-IPV formulation is under review for licensure at the time of publication. See text for details and product descriptions.
+ DT for those with contraindications to pertussis vaccine.
‖ For primary immunization of persons 10–11 years of age and over, one of the three primary series doses (preferably the first) should be Tdap. For persons receiving three doses Td at age 7–10 years, booster dose of Tdap is indicated when aged 11–18 years; an interval of at least 5 years between Td and Tdap is encouraged, but shorter intervals can be used. Booster dose of Td is indicated every 10 years thereafter. Tdap should replace a single dose of Td for adults aged <65 years who have not previously received a dose of Tdap (either in the primary series, as a booster, or for wound management). Only one of two Tdap products (ADACEL® [sanofi pasteur, Swiftwater, Pennsylvania]) is licensed for use in adults. If the person is pregnant and received the last Td vaccination >10 years previously, administer Td during the second or third trimester; if the person received the last Td vaccination in <10 years, administer Tdap during the immediate postpartum period. A one-time administration of 1-dose of Tdap with an interval as short as 2 years from a previous Td vaccination is recommended for postpartum women, close contacts of infants aged <12 months, and all health-care workers with direct patient contact. In certain situations, Td can be deferred during pregnancy and Tdap substituted in the immediate postpartum period, or Tdap can be given instead of Td to a pregnant woman after an informed discussion with the woman.
++ First visit generally at 2 months of age.
¶ Dose 4 of DT indicated at 4–6 years of age unless dose 3 administered at ≥4 years of age. In this instance, dose 4 is not needed. Booster dose of Td is indicated at 11–12 years of age and booster doses of Td are every 10 years thereafter.
§ Dose 5 of DTaP (DTP) or DT indicated at 4–6 years of age unless dose 4 administered at ≥4 years of age. In this instance, dose 5 is not needed. Booster of Tdap is indicated at 11–12 years of age and boosters of Td every 10 years thereafter.
DT, diphtheria and tetanus toxoids for pediatric use; DTaP, diphtheria and tetanus toxoids and acellular pertussis vaccine; DTP, diphtheria and tetanus toxoids and pertussis vaccine; Td, tetanus and diphtheria toxoids for adult use, Tdap, tetanus toxoid reduced diphtheria toxoid and acellular pertussis vaccine for use in adolescents or adults.
From:
(1) Centers for Disease Control and Prevention. Diphtheria, tetanus and pertussis: guidelines for vaccine prophylaxis and other preventive measures. Recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR 40(RR-10):1–28, 1991.
(2) Centers for Disease Control and Prevention. Pertussis vaccination: use of acellular pertussis vaccines among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 46(RR-7):1–25, 1997.
(3) Centers for Disease Control and Prevention. Preventing tetanus, diphtheria, and pertussis among adolescents: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 55(No. RR-3):1–43, 2006.
(4) Centers for Disease Control and Prevention. Preventing tetanus, diphtheria, and pertussis among adults: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 55(No. RR-17):1–33, 2006.
(5) Centers for Disease Control and Prevention. Recommended Adult Immunization Schedule—United States, October 2007–September 2008. MMWR 56:Q1–Q4, 2007.

Preparations should be stored at 2–10°C and generally have a 2-year expiration date. Higher ambient temperatures for 3 days or under do not reduce the potency of the toxoid. Freezing reduces potency, particularly when the toxoid is a component of DTP.[104]

9.2.2. Use of Tetanus Toxoid. Most immunization schedules in developed countries call for two or three doses in the first year of life followed by a reinforcing dose 6 months to 1 year afterward.[105–107] The routine schedule for tetanus immunization in the United States is given in Table 1. In children less than 7 years old, tetanus toxoid is usually combined with diphtheria toxoid and pertussis vaccine as DTaP in various potential combinations with other vaccines. When given as DTaP, three doses starting at 6 weeks to 2 months of age are indicated 1–2 months apart followed by a fourth dose approximately 6–12 months after the third dose. If pertussis vaccine is contraindicated, DT is indicated. In the first year of life, the schedule is identical to the DTaP schedule if substituted. However, if beginning in the second year, only three doses of DT are required for primary immunization: two doses 1–2 months apart followed by a third dose 6–12 months after the second. For developing countries, WHO, with its emphasis on infants and child survival, recommends a total of three doses of DTP early in the first year of life; booster doses can be considered depending on the resources available (Table 2). Ideally, all individuals should receive a total of five doses of tetanus toxoid-containing vaccine in childhood, followed by a sixth

Table 2. Recommendations of the World Health Organization for Immunization with Diphtheria–Tetanus–Pertussis (DTP) and Tetanus and Diphtheria Toxoid (Td) Vaccines Required to Obtain Long-Term Protection Against Tetanus, 2008*

Recommended schedule beginning in infancy	DTP	DTP	DTP	Td	Td	Td
	Before age one or as early as possible after age 6 weeks, with >=4 week intervals			e.g. 4–7 years	e.g. 12–15 years	Early adulthood
Adolescents and adults with no previous immunization	Td	Td	Td	Td	Td	
	As early as possible	At least 4 weeks later	At least 6 months later	At least 1 year later	At least 1 year later	
Pregnant women with no previous immunization (or unreliable immunization information)	Td	Td	Td	Td	Td	
	As early as possible in first pregnancy	At least 4 weeks later	At least 6 months later, or in next pregnancy	At least 1 year later, or in next pregnancy	At least 1 year later, or in next pregnancy	
Pregnant women with 3 childhood DTP doses	Td	Td	Td			
	As early as possible in first pregnancy	At least 4 weeks later	At least 1 year later			
Pregnant women with 4 childhood DTP doses	Td	Td				
	As early as possible in first pregnancy	At least 1 year later				
Supplementary immunization activities in high-risk areas (women of childbearing age)	Td	Td	Td	Td	Td	
	During round 1	During round 2, at least 4 weeks after round 1	During round 3, at least 6 months after round 2	At least 1 year later, (e.g. in next pregnancy)	At least 1 year later, or in next pregnancy	

* Other tetanus-containing combination vaccines can be used as per national immunization schedules.
Source: World Health Organization. Tetanus Vaccine: WHO position paper. Wkly Epidemiol Rec 81:198–208, 2006.

dose in early adulthood to provide added assurance of protection throughout the childbearing years (Table 2).[107]

Protective levels of tetanus antitoxin can be achieved with schedules starting in the newborn period. However, in most countries immunization rarely starts before 2 months of age. In the past, it was thought that passively transferred maternal antitoxin would not interfere with the immune response of the infant. However, most of these studies were done before women had been routinely immunized either as children or as adults. More recent studies in the United States demonstrate that substantial proportions of infants have levels of circulating antitoxin at the time of initiating the DTP series, suggesting that the issue of interference should be reconsidered.[108,109] The need for three doses in the first year of life was supported by a report by Barkin,[108] who demonstrated that substantially higher levels of antitoxin are achieved after three doses in the first year of life compared to two doses. The height of the antitoxin response is directly related to the age at completion of the third dose. There is no good evidence that intervals of 2 months for the first three doses offer any advantage over 1 month. All achieve protective levels regardless of schedule, although antitoxin levels are higher after the 2-month interval probably because these children are older at the time they complete their three-dose schedule. Nevertheless, by the time the fourth dose is indicated (approximately 1 year after the third dose), antitoxin levels are comparable in both groups. Preterm infants appear to respond similarly at the same chronological age as term infants.[105,110]

Unimmunized children 7 years of age or older and adults require a three-dose primary series with two doses 1–2 months apart followed by a third dose 6–12 months after the second (Table 1).[105,106] In the United States, the Td adult preparation that contains a decreased amount of diphtheria toxoid is used (or a single use in adolescence/adulthood of an appropriate Tdap product). The immune response to tetanus

toxoid tends to decrease somewhat with increasing age. In comparative studies, children tend to achieve higher levels of antitoxin than do adults. The elderly tend to make a poorer response to toxoid than do younger adults.[111,112] Nevertheless, almost all children and adults will achieve protective titers after a three-dose primary series.

In developing countries, immunization of adults is directed primarily for pregnant women in order to prevent maternal and neonatal tetanus. Induction of protective levels in mothers provides protection from neonatal tetanus by transfer of antitoxin across the placenta.[8,16,113,114] A minimum of two doses at least 1 month apart has been recommended by the EPI for pregnant women with the last dose at least 2 weeks prior to the estimated date of delivery (Table 2). However, difficulties in finding and vaccinating pregnant women and a desire to eliminate maternal as well as neonatal tetanus have led to efforts in some places to vaccinate all women of childbearing age. In addition, vaccination with only two doses is less than optimally effective and does not provide long-term protection. One dose provides little chance of protection.[8] The schedule listed in Table 2, which recommends a total of five tetanus toxoid doses with the third at least 6 months after the first, has been adopted by the EPI in hopes of inducing sustained levels of circulating antitoxin in all women for life.[107]

Where immunization programs are sufficiently developed, WHO currently recommends that all individuals ideally should receive a total of five doses of tetanus toxoid-containing vaccines in childhood followed by an additional dose in adulthood. Td is recommend at approximately age 4–7 years and again in adolescence (e.g., age 12–15 years) (Table 2). In addition, Td should replace tetanus toxoid for adult vaccination.[107]

After a three-dose primary series, circulating antitoxin remains at protective levels for years. Studies in Denmark demonstrate that 96 and 72% of subjects had levels $\geq$0.01 IU/ml at 13–14 and $\geq$25 years, respectively, after the third dose of a product containing 7–12 Lf.[100] Based on these studies and others, immunization advisory bodies in the United States have recommended routine boosters of a tetanus toxoid-containing preparation every 10 years.[105,106] The booster response is not impaired even with intervals of 20 or more years since the last dose.[115] There is no need to restart a primary series if the schedule has not been followed or booster doses have been missed. Instead, prior doses should be counted and the remaining doses given to finish the three-dose primary series. Missed boosters can be ignored. In the United States, a preschool booster of DTaP is recommended at 4–6 years of age primarily to ensure immunity to pertussis in young schoolaged children. Doses of tetanus toxoid are recommended in the United States at 11–12 years of age and every 10 years thereafter. Since the introduction of Tdap, a single booster is recommended at 11–12 years of age, or for one of the subsequent booster doses in adults when indicated.[116,117]

Tetanus in the United States continues to be a disease primarily of older adults who are also at highest risk for tetanus mortality. To decrease this remaining tetanus burden, the Advisory Committee on Immunization Practices (ACIP) and other advisory committees recommend that for patients aged 50 years, health-care providers annually administer influenza vaccine, and on first contact, should review adult vaccination status, administer tetanus and diphtheria toxoid as indicated, and determine whether a patient has one or more risk factors that indicate the need to receive pneumococcal or other vaccination. In the National Health Interview Survey of 1999, 63% of males 50–64 years of age and 50% $\geq$65 years of age reported having received a tetanus toxoid dose within the prior ten years; among females, the proportions were 51 and 36%, respectively.[118] This compares to 67% of males $\geq$65 years of age who reported receiving influenza vaccine in the previous 12 months and 65% of females.

9.2.3. Adverse Events. Reports of local reactions following tetanus toxoid vary greatly. In general, 50–85% of recipients of booster doses of adsorbed toxoid experience pain or tenderness at the injection site. While 20–30% experience edema and erythema,[119,120] marked swelling occurs in less than 2%. Controlled studies suggest that minor swelling or pain may occur more frequently in individuals receiving Td than among those receiving tetanus toxoid; however, substantial differences in marked swelling have not been demonstrated.[120] Several studies have found that the greater the preexisting antitoxin level, the higher is the incidence of local reactions, which generally begin within 2–8 h of an injection.[119,121,122] There have been reports of massive local swelling, particularly in persons with a history of multiple successive booster doses of toxoid. Such persons are typically found to have high serum antitoxin levels, likely leading to the formation of immune complexes with the deposited toxoid (Arthus reaction).[121,123] Thus, frequent boosters of tetanus toxoid in wound management are discouraged.

Fever can accompany a local response, particularly a marked local reaction.[120] Overall, booster doses of Td are associated with fever in 0.5–7%, with temperature over 39°C being rare.[122,124] Headache and malaise are unusual.

In 1994, the Institute of Medicine published an extensive review of adverse events following vaccination.[125] This review suggested that the evidence favors a causal relation between tetanus toxoid administration and Guillain–Barré syndrome, as well as brachial neuritis. The evidence was considered insufficient to assess a causal relationship between tetanus toxoid and mononeuropathy. Subsequent

population studies do not support a causal role for tetanus toxoid in GBS.[126]

Anaphylactic reactions to purified tetanus toxoid appear to be rare.[125] In the United States, passive surveillance in 1991–1995 for serious allergic reactions including laryngospasm, bronchospasm, or anaphylaxis yielded a rate of 1.6 serious allergic reactions reported per million doses of publicly distributed Td.[127] Skin testing has been urged in management of patients with a history suggestive of such a reaction.[128] Interpretation of some skin test results should take into account some expected nonspecificity of skin test reactions, particularly since only adsorbed products are widely available.

9.2.4. Wound Management. An individual who presents for medical care with any type of wound should be evaluated for potential tetanus prophylaxis. Removal of foreign bodies and debridement of devitalized tissue in a timely fashion are imperative; if necessary, drainage and irrigation should be performed.[93,94] Recommendations for the use of tetanus toxoid and TIG in the United States for tetanus prophylaxis in the management of wounds have been made by the ACIP and agree with those of the American Academy of Pediatrics and the Committee on Trauma of the American College of Surgeons.[93,105,106] The recommendations of the ACIP are given in Table 3.

Although any wound can potentially give rise to tetanus infection, uncomplicated wounds are considered to have a low likelihood both of contamination by tetanus spores and of leading to an environment that would support germination of spores. For persons with this category of wounds, Td or Tdap is recommended if the patient has received less than three doses of adsorbed toxoid in the past or it has been more than 10 years since the previous toxoid dose; TIG administration is not necessary. Such individuals respond briskly to subsequent doses of tetanus toxoid generally within 4 days.[129]

Most patients who had received three doses of fluid toxoid in the past, and rarely individuals who received three doses of adsorbed toxoid, may have circulating antitoxin levels less than 0.01 IU/ml after 5 years.[16,130] Therefore, it is recommended that persons with wounds that are of higher risk of contamination (wounds that are neither minor nor clean) receive a dose of toxoid if more than 5 years have elapsed since the last dose. Because fluid toxoid is no longer used for tetanus prophylaxis in the United States, the rationale for this recommendation is becoming less relevant. Individuals with wounds that are neither minor nor clean are also potential candidates for simultaneous passive immunization with TIG if the immunization history indicates fewer than three toxoid doses. When administered at a separate site, TIG does not interfere with the immune response to adsorbed tetanus toxoid. An intramuscular dose of 250 units (1 ml) is recommended by the AAP and ACIP;[105,106] a dose of 500 units is recommended by some for patients with more serious wounds, who presented with delay or who weigh more than the average adult.[93,131,132]

Based on the history of care, almost all of the tetanus cases with wounds in the United States might have been prevented with appropriate wound management. Of the 992 patients in the United States during 1972–1989 with acute wounds, tetanus toxoid was given as prophylaxis in wound management to only 29%; of these, 77% received toxoid within 3 days of injury.[49] Tetanus toxoid alone is not sufficient in patients who have never received at least a three-dose primary series. Most of these individuals were also potential candidates for, but did not receive, TIG. Thirty-three percent of the 992 patients with acute wounds reportedly underwent debridement after injury and before onset of tetanus symptoms. Of these 327 patients, 93% did not receive the recommended Td with or without TIG based on their individual immunization histories. Although underadministration of tetanus prophylaxis following wounds is the predominant

Table 3. Summary Guide to Tetanus Prophylaxis in Routine Wound Management, United States, 2008

History of adsorbed tetanus toxoid (doses)	Clean, minor wounds		All other wounds*	
	TTCV+	TIG	TTCV+	TIG
Unknown or <3	Yes	No	Yes	Yes
≥3**	No §	No	No†	No

* Including, but not limited to, wounds contaminated with dirt, feces, soil, saliva; puncture wounds; avulsions; and wounds resulting from missiles, crushing, burns and frostbite.

+ TTCV, Tetanus toxoid-containing vaccine. For children <7 years old, DTaP (DT, if pertussis vaccine is contraindicated) is preferred to TT alone. Whole-cell DTP may be used instead of DTaP as indicated, or in other countries. For children 7–9 years of age, Td is preferred to TT alone. Tdap is preferred to Td for persons aged 10–64 years who have never received Tdap, and Td is preferred to TT for persons aged 10–64 years who received Tdap previously or when Tdap is not available. Only one of two Tdap products (ADACEL® [sanofi pasteur, Swiftwater, Pennsylvania]) is licensed for use in adults. (If TT and TIG are both used, Tetanus toxoid adsorbed rather than tetanus toxoid for booster use only [fluid vaccine] should be used).

** If only 3 doses of *fluid* toxoid have been received, then a fourth dose of toxoid, preferably an adsorbed toxoid, should be given.

§ Yes, if more than 10 years since last dose.

† Yes, if more than 5 years since last dose. (More frequent boosters are not needed and can accentuate side effects.)

DT, diphtheria and tetanus toxoids for pediatric use; DTP, diphtheria and tetanus toxoids and pertussis vaccine; Td, tetanus and diphtheria toxoids for adult use; TT, tetanus toxoid.

From:

(1) Centers for Disease Control and Prevention. Diphtheria, tetanus and pertussis: guidelines for vaccine prophylaxis and other preventive measures. Recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR 40 (No. RR-10), 1991.

(2) Centers for Disease Control and Prevention. Preventing tetanus, diphtheria, and pertussis among adolescents: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccines. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 55(No. RR-3):1–43, 2006.

(3) Centers for Disease Control and Prevention. Preventing tetanus, diphtheria, and pertussis among adults: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 55(No. RR-17):1–33, 2006.

history in reported tetantus patients with wounds, underadministration of tetanus prophylaxis following wounds overall appears to be the exception rather than the rule. Three studies indicated that when patients with wounds sought care in the United States, 1–35% received less than the recommended prophylactic measures of Td with or without TIG indicated by their wound and immunization history and 8–17% received more than recommended.[133–135]

9.3. Antimicrobials

As a substitute for or adjunct to equine antitoxin prior to wide availability of TIG, antibiotics were once frequently used for tetanus prophylaxis in wound management. Antimicrobials may cause complications and have been known to fail. Antimicrobials have not been proven to have superior or equal efficacy to antitoxin and are not currently recommended for tetanus prophylaxis.[94,136] There are some data to suggest antibiotic ointment application to the umbilical stump may prevent neonatal tetanus.[66,67]

9.4. Summary

Tetanus is an toxin-mediated infectious but noncommunicable disease. The spores of *C. tetani* are ubiquitous in nature and when deposited under the epithelium of the skin can germinate, given anaerobic conditions, leading to the production of tetanospasmin. The toxin travels to the CNS along peripheral nerves and causes disease by presynaptic interference with release of inhibitory neurotransmitters, leading to disinhibition of spinal cord reflex arcs and accumulation of excitatory stimulation. This results in the tetanospasm, a generalized muscular spasm. Death-to-case ratios vary from 10 to 75%, depending on care, immunization status, age, and other risk factors.

The disease can be prevented by appropriate attention to routine immunization and wound management. Tetanus toxoid is one of the most effective immunogens known, with essentially all recipients of a primary series protected for at least 5–10 years and often 25 years. Since wounds are the most frequent predisposing cause of tetanus outside the neonatal period, special attention should be paid to (1) wound cleansing and debridement to remove both spores and devitalized tissue and (2) providing protective levels of circulating antitoxin including both passive immunization to achieve protection rapidly and active immunization to increase levels rapidly in previously primed persons. Completion of a primary series will lead to sustained protection and prevent cases not associated with acute injuries; appropriate routine immunization would circumvent the need for passive immunization following wounds. It is particularly important to review the immunization history of elderly patients to ensure completion of primary immunization and routine boosting.

Tetanus can occur within the first month of life in infants born to unimmunized women followed by infection by *C. tetani.* Cases are often associated with unsterile conditions of childbirth and with unhygienic cultural rituals involving the umbilical stump. Although neonatal tetanus was once responsible for an estimated 1.2 million deaths per year, because of the increasing immunization coverage of pregnant women and women of childbearing age, targeted mass immunization in high-risk populations, and the increasing prevalence of clean deliveries, its annual burden has been reduced to less than 180,000 neonatal tetanus deaths. Maternal and neonatal tetanus is targeted for elimination through continued efforts to reach all populations.

10. Unresolved Problems

Although the disease is becoming uncommon, the major problem in tetanus prevention today continues to be the difficulty in delivering routine immunization to those in need. In 2004, an estimated 40 million pregnant women in developing countries were still in need of immunization against birth-associated tetanus and an estimated 180,000 infants die of neonatal tetanus annually.[41,107] The vast majority of these deaths occur in countries with limited health services where most women do not have ready access to prenatal care. The goal of maternal and neonatal tetanus elimination made in 1989 was renewed in 2000 with a target of 2005; the advances made since 2000 have been primarily because of campaigns to vaccinate pregnant women in the highest risk areas.[107,137] In all developing countries, most children are born outside of a hospital; in the countries at highest risk, there needs to be greater emphasis on prenatal care, attended delivery and potentially vaccination of women of childbearing age if maternal and neonatal tetanus is to be eliminated as a significant public health problem. Every contact of a susceptible female of childbearing age with the health-care system could be taken as an opportunity to update her tetanus immunization status. In addition, there is a continuing challenge to sustain progress made in immunizing women and in increasing the prevalence of clean deliveries.

In 1999, WHO instituted a validation process to monitor progress toward the goal of sustainable elimination of maternal and neonatal tetanus for each country at risk.[41] Using that process, 11 of the 57 countries remaining at risk

for maternal and neonatal tetanus in 1995 have been validated as having eliminated it. As of January 2008, more than 145 countries and areas have succeeded in reaching the WHO definition of elimination. Based on this progress, WHO estimates that an additional eight countries will reach and validate elimination by the end of 2008.[138]

Routine immunization remains incomplete; about 27 million children a year did not complete their primary tetanus immunization series.[107] With strengthening of child immunization services and with booster immunizations becoming progressively routine (to ideally vaccinate every child five times by the age of 15 years), tetanus in all ages will become increasingly rare.

In the United States, the limited remaining morbidity and mortality from tetanus are predominantly in the elderly who have never completed primary immunization. Serological surveys suggest that substantial majorities of these populations lack protection.[22] Greater efforts are needed by practitioners to review the immunization status of their elderly patients and offer Td as needed.

Proper wound management could still prevent a significant proportion of current tetanus cases. All health-care providers, particularly in emergency departments, need to be familiar with the recommendations for tetanus prophylaxis in wound care, including administration of passive immunization when applicable to persons who are unimmunized or whose immunization status is unknown. However, since chronic conditions and mild injuries are also associated with tetanus, the best approach to tetanus prevention is universal routine immunization and boosting.

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CHAPTER 39

Toxic Shock Syndrome (Staphylococcal)

Arthur L. Reingold

1. Introduction

Staphylococcal toxic shock syndrome (TSS) is an acute, multisystem febrile illness caused by *Staphylococcus aureus*. A similar illness caused by group A streptococcal infections is discussed in Chapter 34. The accepted criteria for confirming a case of TSS include fever, hypotension, a diffuse erythematous macular rash, subsequent desquamation, evidence of multisystem involvement, and lack of evidence of another likely cause of the illness (Table 1).

2. Historical Background

TSS was first described as such in 1978 by Todd et al.[1] However, cases of what we now believe to have been TSS have been reported in the medical literature since at least 1927 as "staphylococcal scarlet fever" or "staphylococcal scarlatina."[2,3] In addition, a number of patients reported in the medical literature in the 1970s as having adult Kawasaki disease probably had TSS.[4] The association between illness and focal infection with *S. aureus* was, by definition, apparent in early reports of staphylococcal scarlet fever, but was reinforced by the findings of Todd et al.[1] and later by the findings of other investigators.[5,6]

TSS achieved notoriety in 1980 when numerous cases were recognized and an association between illness (in women), menstruation, and tampon use was demonstrated.[7,8] While the early case reports of staphylococcal scarlet fever and the report by Todd et al.[1] clearly showed that TSS occurred in small children, men, and women who were not menstruating, most (but by no means all) of the cases initially recognized and reported in late 1979 and early 1980 were in menstruating women,[8–10] leading to the frequent misperception among the general public and many physicians that TSS occurred only in association with tampon use (hence, "the tampon disease"). This misperception undoubtedly led to subsequent biases in the diagnosing (and probably reporting) of TSS cases. However, later studies designed to eliminate such biases have shown that TSS does, in fact, occur disproportionately in menstruating women,[11,12] while case-control studies demonstrating an association between the risk of developing TSS during menstruation and tampon use preceded (indeed, led to) the introduction of bias concerning the relationship between tampon use and menstrual TSS.[5,6]

Follow-up studies demonstrated that the risk of developing tampon-related menstrual TSS varies with the absorbency, chemical composition, and oxygen content of the tampon,[13–16] although the relative importance of these and other tampon characteristics in determining that risk remains uncertain. As a result of both epidemiological and in vitro laboratory studies, the formulation of available tampons changed dramatically in the early 1980s, such that absorbencies were substantially lower and chemical composition was less varied across brands and styles. Studies in the late 1980s demonstrated that the incidence of TSS, particularly menstrual TSS, rose and fell in parallel with the absorbency of tampons,[17] but that the risk of developing menstrual TSS continued to vary directly with tampon absorbency, despite the changes in tampon formulation.[18]

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Table 1. Case Definition of Toxic Shock Syndrome

Fever: temperature $\geq$ 38.9 °C (102 °F)
Rash: diffuse macular erythroderma
Desquamation: 1–3 weeks after onset of illness
Hypotension: systolic blood pressure $\leq$ 90 mmHg for adults or below fifth percentile by age for children under 16 years of age, orthostatic drop in diastolic blood pressure $\geq$ 15 mmHg from lying to sitting, orthostatic syncope, or orthostatic dizziness
Multisystem involvement: three or more of the following:
Gastrointestinal: vomiting or diarrhea at onset of illness
Muscular: severe myalgia or creatine phosphokinase level at least twice the upper limit of normal for laboratory
Renal: blood urea nitrogen or creatinine at least twice the upper limit of normal for laboratory or urinary sediment with pyuria ($\geq$ 5 leukocytes per high-power field) in the absence of urinary tract infection
Hepatic: total bilirubin, serum aspartate transaminase, or serum alanine transaminase at least twice the upper limit of normal for laboratory
Hematologic: platelets $<$ 100,000 mm^3
Central nervous system: disorientation or alterations in consciousness without focal neurological signs when fever and hypotension are absent
Negative results on the following tests, if obtained:
Blood, throat, or cerebrospinal fluid cultures (cultures may be positive for *Staphylococcus aureus*)
Rise in titer to Rocky Mountain spotted fever, leptospirosis, or rubeola

3. Methodology

3.1. Sources of Mortality Data

Mortality rates for TSS have not been reported directly, but can be estimated from reported incidence rates and case-fatality ratios.

3.2. Sources of Morbidity Data

Surveillance for TSS began in a few states in late 1979 and in other states and nationally in early 1980. Since that time, TSS has been made a reportable disease in most states. However, the level of intensity of surveillance activities has varied markedly between and within states. Thus, a few states established active surveillance for TSS for brief periods of time, while others did little to stimulate the diagnosis and reporting of cases. As a result, the completeness of diagnosing and reporting TSS cases undoubtedly has been inconsistent between states and over time. However, data from a national hospital discharge survey indicated that reporting of cases in the 1980s, while incomplete and variable by region, was not biased dramatically insofar as the age, race, sex, or menstrual status of the patients was concerned.[19] Hospital record review studies, in which both diagnosed and previously undiagnosed cases of TSS were ascertained in a consistent fashion, so as to minimize or eliminate both diagnostic and reporting biases, also have been conducted.[11,12,17,20,21] These studies demonstrated that, by and large, the patient characteristics and temporal trends observed in data collected through the largely passive network of TSS surveillance reflected true variation in the incidence of TSS by age, sex, race, and menstrual status. These same studies, taken together, demonstrated that at least some of the apparent geographic variation in the incidence of TSS in the United States in the 1980s was real. More recent information concerning the epidemiologic features of TSS comes almost entirely from the passive national surveillance system, as few epidemiologic studies of TSS have occurred since the 1980s.

3.3. Surveys

Numerous small surveys have demonstrated that many asymptomatic individuals carry, in the nasopharynx and/or vagina, strains of *S. aureus* that produce TSS toxin-1 (TSST-1), the toxin believed to be responsible for most TSS cases.[22–26] Similarly, large serosurveys have shown that antibodies to TSST-1 or to a cross-reacting antigen are extremely common.[22,25,27,28]

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organism. While recovery of *S. aureus* from the vagina or another site of infection is not one of the criteria of the TSS case definition, it is possible in most TSS cases if appropriate specimens are obtained before antimicrobial therapy is initiated.[5,6,29,30] *S. aureus* grows readily on most standard culture media and is readily identifiable by any clinical microbiology laboratory within 2 or 3 days. Testing of *S. aureus* strains for production of TSST-1, however, is performed in only a few research laboratories. Hence, the results of such testing are not readily available during the acute illness and are not of value in treating patients suspected of having TSS. Furthermore, because both *S. aureus* in general and TSST-1 producing strains of *S. aureus* in particular can be recovered from many patients without the clinical features of TSS and from asymptomatic individuals, microbiological results cannot and do not prove that a given patient has TSS.

3.4.2. Serological and Immunologic Diagnostic Methods. A variety of serological and immunologic techniques have been used to test *S. aureus* strains for production of TSST-1. As noted above, these tests are not available outside a few research laboratories. It is possible to detect TSST-1 in clinical specimens,[31,32] but these assays are not generally available. Antibodies to TSST-1 can be measured using solid-phase radioimmunoassay and other techniques. However, most healthy individuals have

detectable anti-TSST-1 antibodies.[22,25,27,28] Furthermore, some patients with TSS have demonstrable anti-TSST-1 antibodies at the time of onset, and many patients without such antibodies at the time of onset do not demonstrate an antibody rise in response to their illness.[27,33] Thus, testing for anti-TSST-1 antibodies (which is not available except in one or two research laboratories, in any event) is of limited value in confirming the diagnosis of TSS, although it has been argued that the absence of detectable antibodies at the time of onset supports the diagnosis of TSS.

4. Biological Characteristics of the Organism

As noted above, there is convincing evidence that *S. aureus* is the cause of TSS. In patients with menstrual TSS, *S. aureus* can be recovered from the vagina and/or cervix in 95–100% of cases (usually as a heavy growth), but in only 5–15% of healthy control women.[5,24,34–39] In patients with nonmenstrual TSS associated with a focal wound, *S. aureus* is typically the only organism found in the lesion.[29,30] Furthermore, experimental studies demonstrate that TSS-associated *S. aureus* strains can cause a similar illness in rabbits.

Similarly, there is strong evidence that the ability to make TSST-1, previously known as pyrogenic exotoxin C,[40] staphylococcal enterotoxin F,[41] and several other names, is characteristic of, although not universal among, TSS-associated *S. aureus* strains. Thus, 90–100% of *S. aureus* isolates recovered from the vagina, cervix, or used tampon in menstrual TSS cases produce TSST-1, compared with only 10–20% of vaginal or nasopharyngeal isolates from healthy controls.(26,40–43) On the other hand, only 60–70% of *S. aureus* strains recovered from normally sterile sites in patients with nonmenstrual TSS produce TSST-1,[44–46] suggesting that other staphylococcal toxins, particularly staphylococcal enterotoxin B (SEB), are capable of inducing a clinically indistinguishable syndrome. Two studies of historical strains of *S. aureus* demonstrated that the proportion of strains capable of making TSST-1 has changed over time and was generally higher in the mid to late 1970s than in earlier time periods.[42,47] Interestingly, that proportion appears to have declined somewhat in the early 1980s, when the incidence of TSS was peaking. More recent data concerning the proportion of *S. aureus* strains that make TSST-1 have not been published.

TSS-associated *S. aureus* strains also have been characterized phenotypically with respect to a number of other properties, including phage type, antimicrobial susceptibility, resistance to heavy metals, production or activity of various enzymes, and presence of plasmids and bacteriophages. The picture that emerges with regard to these characteristics, while consistent, is by no means invariable or unique. A higher proportion of TSS-related *S. aureus* strains are lysed by phase types 29 and/or 52 (58–82%), as compared to only 12–28% of control strains.[42,43,48] Similarly, TSS-associated strains generally are resistant to penicillin (and ampicillin), arsenate, and cadmium, while being susceptible to β-lactamase-resistant antimicrobial agents, most other commonly tested antimicrobial agents, bacteriocins, and mercury.[6,49,50] Other characteristics that appear to distinguish these strains from other *S. aureus* strains include decreased production of hemolysin, lipase, and nuclease;[49,51] tryptophan auxotypy;[52] decreased lethality in chick embryos;[49] increased pigment production;[53] and increased casein proteolysis.[53] TSS-associated strains also have been reported to be less likely to carry plasmids and more likely to carry lysogenic bacteriophage than control strains. There is controversy over whether or not the gene coding for TSST-1 can be transferred by lysogeny.[54,55]

It should be noted that most of the strains examined in the above studies were recovered from the genital tract in menstrual TSS cases. Thus, the results are not necessarily applicable to *S. aureus* strains associated with nonmenstrual TSS, and there is some evidence to suggest that such strains, recovered from normally sterile sites in patients with nonmenstrual TSS, are less likely to be lysed by phage types 29 and/or 52 than strains from menstrual TSS cases.[44] At the same time, as noted above, they also are less likely to make TSST-1.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Carriage of *S. aureus* on the skin and in the nasopharynx and vagina is very common. Numerous cross-sectional studies have demonstrated that 30–40% of individudals carry *S. aureus* in the nasopharynx and 5–15% of women carry *S. aureus* in the vagina.[22–24,34–39] The corresponding figures for TSST-1-producing *S. aureus* are 5–15% (nasopharynx) and 1–5%(vagina). Thus, carriage of *S. aureus* strains believed to be capable of causing TSS is also very common.

In contrast, TSS is a rare disease. After it became a notifiable disease in 1983, the number of cases reported annually in the United States initially ranged from 400 to 500. More recently, approximately 100–150 cases have been reported annually to the Centers for Disease Control and Prevention (CDC).[56] The most reliable estimates of incidence rates come from hospital-based record review studies. In these studies, both diagnosed and previously undiagnosed cases of TSS were ascertained in an unbiased way by reviewing

thousands of medical records of hospitalized patients with one of a long list of discharge diagnoses likely to be indicative of misdiagnosed cases of TSS. In one such study in Colorado, the annual incidence of TSS in women between the ages of 10 and 30 years was 15.8/100,000 in 1980.[12] In a similar study in California, the incidence rate in women between the ages of 15 and 34 years was only 2.4/100,000 in 1980.[11] The incidence rate in men of the same age group in the latter study was consistently less than 0.5/100,000 in all of the years studied.

Initial estimates of the incidence of diagnosed TSS derived from statewide surveillance systems established in late 1979 or early 1980. The states with the most aggressive case-finding methods reported annual incidence rates, at that time, of 6.2/100,000 menstruation women (Wisconsin),[5] 8.9/100,000 menstruating women (Minnesota),[57] and 14.4/100,000 females 10–49 years of age (Utah).[58] An overall estimate of 0.8/100,00 total population of hospitalized, diagnosed TSS in the United States in 1981 and 1982 was derived from a national hospital discharge survey.[19] More recent estimates of the incidence of TSS, based on admittedly incomplete passive surveillance, are even lower.[56] While TSS has been documented in numerous other countries, no estimates of incidence rates for other countries are available.

The discrepancy between the frequency of colonization and/or infection with TSST-1-producing *S. aureus* and the rarity of TSS is thought to be due to the fact that most individuals have detectable anti-TSST-1 antibodies. By age 30 years, more than 95% of men and women have such antibodies.[28] The origin of these antibodies is unknown.

5.2. Epidemic Behavior and Contagiousness

Because TSS increased dramatically in incidence in the United States beginning in 1979 in comparison with previous years,[11,12,20] it would be correct to say that an epidemic of TSS occurred at that time. TSS does not occur, however, in explosive epidemics in the same way that dengue and meningococcal disease do, although strains of *S. aureus* that produce TSST-1 are, like other *S. aureus* strains, transmitted readily by person-to-person spread.

5.3. Geographic Distribution

5.3.1. United States. Cases of TSS have been reported in all 50 states and the District of Columbia, but the incidence of reported cases has varied substantially between states and regions.[9,10,56] Variation in the completeness of diagnosis and reporting of cases undoubtedly accounts for some of the observed differences, but there is substantial evidence that at least some of the observed differences are real. For example, a study of hospital discharge data in which differences in the reporting of cases could not have been a factor showed that the overall annual incidence of hospitalized cases varied by region between 0.24 and 1.43/100,000 in 1981–1982.[19] In this study, however, potentially large differences in the completeness with which TSS cases were diagnosed and different standards for hospitalizing patients suspected of having TSS could not be ruled out. More convincing evidence for true geographic differences in incidence rates comes from the virtually identical hospital record review studies conducted in Colorado and northern California, in which variation in the diagnosing and reporting of cases was largely or completely eliminated.[11,12] As noted above, the incidence of TSS in 1980 in females 10–30 years of age was 15.8/100,000 in Colorado, but only 2.4/100,000 females 15–34 years of age in northern California. However, a prospective study employing active surveillance for TSS in five states (Missouri, New Jersey, Oklahoma, Tennessee, and Washington) and one large county (Los Angeles) showed that in 1986 the incidence of menstrual TSS was in the range of 1/100,000 females 15–44 years of age in all six study areas.[59]

Studies of *S. aureus* strains from the United States show no geographic differences in what proportion make TSST-1.[47] Similarly, anti-TSST-1 antibodies are found in similar proportions of healthy individuals in different parts of the United States.

5.3.2. Other Countries. Documented cases of TSS have been reported from Canada, most of western Europe, Australia, New Zealand, Japan, Israel, South Africa, and elsewhere. No information concerning incidence rates of TSS outside of the United States is available. However, the proportion of cases in other countries associated with menstruation and tampon use appears to be substantially lower than in the United States, in keeping with the fact that tampon use in general is less frequent in other countries and superabsorbent tampons are less widely used.

5.4. Temporal Distribution

Substantial controversy has surrounded the interpretation of observed changes over time in the diagnosis and reporting of TSS cases. Data from the passive national surveillance system suggested that the number of cases began to rise in 1978, peaked in 1980, and then declined and leveled off, with virtually all of the observed differences being due to changes in the number of menstrual TSS cases reported[9,10] (Figure 1). While this pattern was also observed in some individual states employing vigorous case-finding methods

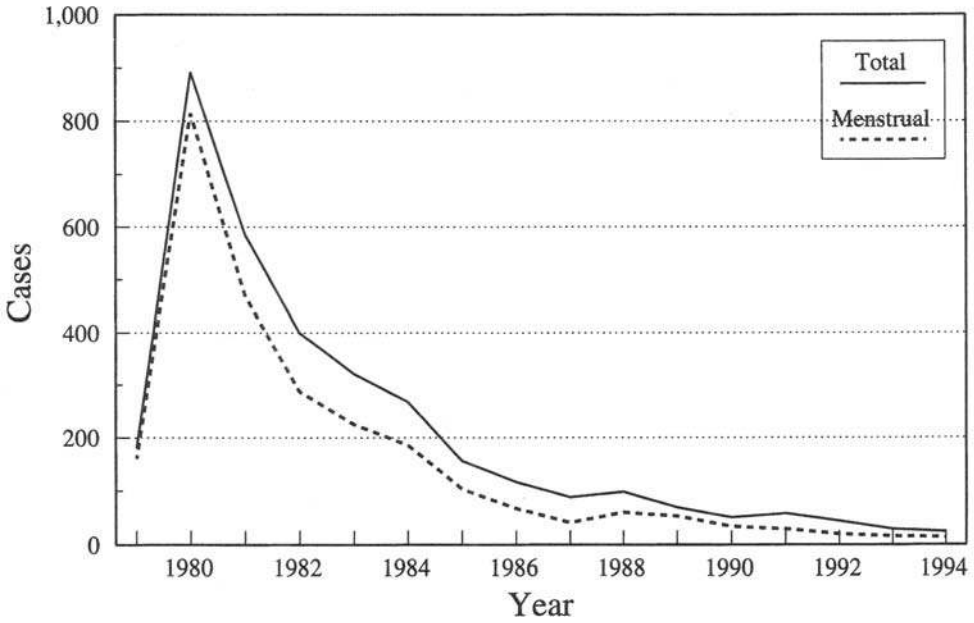


Figure 1. Reported cases of toxic shock syndrome, United States, 1979–1994.

(e.g., Utah and Wisconsin), a different pattern was seen in Minnesota, where no decline in the number of cases was observed in 1981.[60] Because of the documented impact of publicity on reporting of TSS cases and the undoubted fluctuation over time in the likelihood that cases would be diagnosed and/or reported, the results of studies that eliminate or minimize these influences are important in interpreting temporal trends.

While the three published hospital record review studies all suffer from having a relatively small number of cases of TSS to analyze statistically, the results of all three studies are consistent. In the California study, the incidence of TSS in women increased consistently through 1980, fell somewhat in 1981 and 1982, and then increased again in 1983, while the incidence in men remained consistently low (Figure 2). In the Colorado study, the results were similar except that the decrease in 1981 compared with 1980 was sharper (Figure 3). The similarity of the pattern in Colorado is even more apparent if cases meeting only the authors' proposed screening definition for TSS and not the more rigorous collaborative case definition are removed.[61] Similar trends are seen in the study from Cincinnati, although incidence rates cannot be estimated in this study.[20]

Thus, there is convincing evidence that hospitalized cases of TSS in females of menstrual age increased in the late 1970s, irrespective of any changes in the recognition and reporting of the disease. A similar increase was not apparent among men. There is also some evidence that this upward trend in the incidence of TSS through 1980 was reversed in several geographic areas, at least temporarily, in 1981.

5.5. Age

TSS can occur in individuals of all ages and has been documented in a newborn baby and in patients up to 80 years of age. However, data from both passive and active surveillance systems and from the California record review study indicate that younger women are at greater risk of developing TSS than older women. Of cases associated with menstruation reported nationally, almost 60% have been in women 15–24 years of age, compared with only 25% in women 25–34 years of age.[9,10] Cases in women 35–44 years of age are even less common. Furthermore, the highest age-specific incidence rates have been observed consistently in women 15–19 or 15–24 years of age. Thus, in the California record review study, the annual incidence rate was 2.6/100,000 women 15–19 years of age compared with rates of 0.8–1.4/100,000 among women 20–24, 25–29, and 30–34 years of age.[11] Similarly, in Minnesota the annual incidence of menstrual TSS among women 15–24 years of age was 13.7/100,000 compared with rates of 2.3 in those <15 years of age and 6.6 in those ≥ 25 years of age.[56] The age distribution of TSS cases unassociated with menstruation is more uniform, especially if cases in postpartum women are excluded.[30]

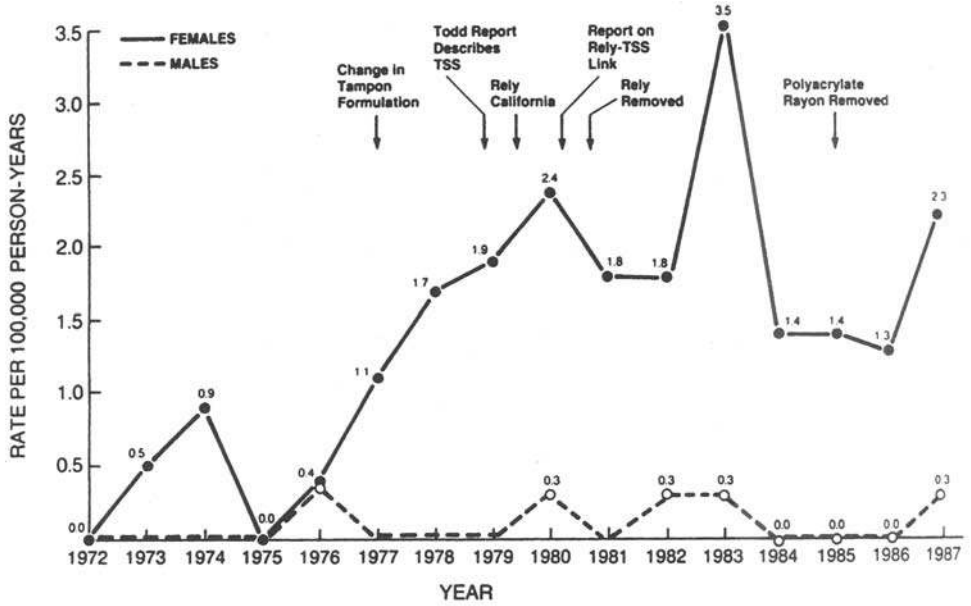


Figure 2. Incidence of hospitalized toxic shock syndrome cases in males (*dashed line*) and females (*solid line*), aged 15–34 years, northern California Kaiser-Permanente Medical Care Program, 1972–1987. Reproduced from Petitti et al.[21] with permission.

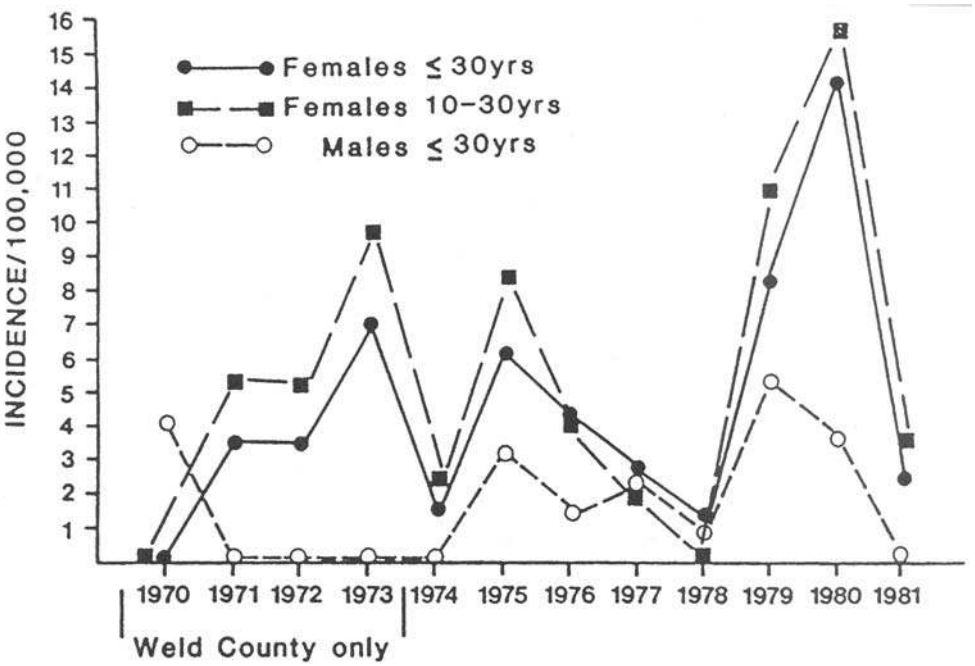


Figure 3. Annual incidence per 100,000 population of toxic shock syndrome in hospitalized patients ≤30 years of age meeting either the strict or the screening case definition in two Colorado counties, 1970–1981. Reproduced from Todd et al.[12] with permission.

5.6. Sex

All available evidence clearly indicates that TSS is much more common among women of menstrual age than among men of the same age. Of US cases reported through passive surveillance, 95% have been in women and 5% in men.[9,10] In the California record review study, the overall incidence of TSS in women 15–34 years of age during the time period 1972–1983 was 15 times that in men of the same age (1.5 vs. 0.1/100,000) person years.[11] This marked difference in incidence rates between men and women undoubtedly relates primarily to the fact that most cases of TSS are associated with menstruation and tampon use. What appears to be an increased risk of TSS during the postpartum interval and the apparent association between TSS and the use of barrier contraception probably contribute further to this pattern.[29,30,62] The incidence of TSS associated with other types of staphylococcal infections (e.g., surgical wound infections, cutaneous and subcutaneous lesions) appears to be similar in men and women.[29,30,59]

5.7. Race

Although it has been apparent since at least 1980 that TSS occurs in individuals of all racial groups,[8] the over-whelming majority (93–97%) of reported cases have been in whites, who make up only 80–85% of the US population.[9,10] Likely explanations for this discrepancy fall into two categories: biases in the diagnosis and reporting of cases on the one hand and true racial differences in either susceptibility to TSS or exposure to risk factors on the other. It has been postulated that increased difficulty in recognizing the rash on dark-skinned individuals, poorer access of minority groups to medical care, and the relative paucity of individuals of races other than white in areas with active TSS research efforts have all contributed to the observed racial distribution of cases. However, data from the California record review study indicate that in the 15–34 age group TSS does indeed disproportionately affect whites.[11] All of the 54 definite cases (most of which are related to menstruation) found in that study were in whites, while only 81% of the population at risk was white ($p < 0.05$; Fisher's exact test, two-tailed).

It has been noted that the racial distribution of patients with nonmenstrual TSS (87% white) more closely resembles the racial distribution of the US population than does the racial distribution of patients with menstrual TSS (98% white).[10,59] Taken together with studies demonstrating that young white women use tampons far more often than do comparably aged women of other racial groups,[63–66] these results suggest that observed race-specific differences in incidence rates are due, at least in part, to different levels of exposure to an important risk factor for developing TSS during menstruation.

5.8. Occupation

There is no evidence to suggest that any given occupational group, including health care providers, is at increased risk of developing TSS.

5.9. Occurrence in Different Settings

As noted below, transmission of strains of *S. aureus* capable of causing TSS has been demonstrated in the hospital setting.[67–69] There is also evidence of spread of these strains and occasional clustering of cases in households and in military installations (CDC, unpublished observations).

5.10. Socioeconomic Factors

It is unclear to what extent the marked racial variation in tampon use, especially among adolescents, reflects socioeconomic rather than racial differences. Other socioeconomic factors have not been noted to play a role in TSS.

5.11. Other Factors

5.11.1. Menstrual TSS. Numerous case–control studies conducted in 1980–1981 examined risk factors for developing TSS during menstruation (Table 2). These studies consistently found that tampon use increased the risk of menstrual TSS (the Oregon study, with its small number of cases, while not finding an association between menstrual TSS and tampon use in general, did find an association with a particular brand of tampon).[5,6,15,16,58,70] Included among these studies are two performed before any information concerning this association had appeared in the medical literature or lay press.[5,6] A study comparing tampon use among women with menstrual TSS in 1983 and 1984 with tampon usage patterns ascertained via a national survey found evidence of a continuing increased risk of menstrual TSS among tampon users.[13] Furthermore, a multistate case–control study of menstrual TSS cases with onset in 1986–1987 documented that this association persisted at that time.[18] No additional case–control studies have been conducted since that time.

Two early case–control studies demonstrated that the risk of menstrual TSS varied with tampon brand and/or style (i.e., absorbency), suggesting that risk was a function of tampon absorbency and/or chemical composition.[15,16] The two more recent studies document clearly that risk of menstrual

Table 2. Risk Factors for Menstrual TSS

						Tampons			
Case onset dates	Date of study	Geographic area	Source of controls	No. cases	No. controls	% Cases	% Controls	Odds ratio[a]	*p* value
9/75–6/80	1980	Wisconsin	Clinic	35	105	97	76	10.6*	<0.01
12/76–6/80	6/80	USA(CDC)	Friend	52	52	100	85	20.1*	<0.05
7/80–8/80	9/80	USA(CDC)	Friend	50	150	100	83	20.5*	<0.01
1/76–8/80	5/80–8/80	Utah	Neighbor	29	91	100	77	18.0*	0.012
10/79–9/80	10/80–11/80	Minnesota, Wisconsin, Iowa	Neighbor	76	152	99	81	18.0	<0.001
12/79–11/80	1/81–3/81	Oregon	Friend		18	100	78	11.5*	NS[b]
			Clinic		18		89	5.6*	NS
1/86–6/87	1/86–8/87	Los Angeles County, Missouri, New Jersey, Oklahoma, Tennessee, Washington	Friend	180	185		71	19	<0.01
			Neighbor		187		60	48	<0.01

	Rely brand tampons			Absorbency		Oral contraceptives				
% Cases	% Controls	Relative risk	*p* value	Odds ratio in multivariate model	*p* value	% cases	% controls	Odds ratio	*p* value	References
	NA[c]		NS	NA		17	36	0.36*	NS	(5)
33	27	NA	NS	NA		4	7	0.48*	NS	(6)
71	26	7.7	<0.0001	1.0	NS	NA	NA	NA		(16)
63	24	6.1	<0.0005	NA		3	11	0.29*	NS	(113)
53	29	2.5	0.005	3.2–10.4	0.01	12	20	0.55*	0.05	(15)
67	17	10.0*	<0.05	NA						(114)
	6	34.0*	<0.05	1.34/g		25	24	1.1	NS	(18)

[a]If not reported in reference, crude odds ratio estimated disregarding matching; 0.5 added to all cells in tables with a 0 value; estimated values indicated with an asterik.
[b]NS, not significant.
[c]NA, data not available.

TSS is directly correlated with measured in vitro tampon absorbency,[13,18] independent of chemical composition, but that chemical composition is also a factor.[13] It is interesting to note that the correlation with tampon absorbency has persisted, despite the major alterations in chemical composition and marked decreases in the absorbencies of available tampons that have occurred since 1980. A reanalysis of data from earlier studies has suggested that the oxygen content of tampons is a better predictor of the risk of menstrual TSS than either chemical composition or absorbency.[14] In vitro studies examining the effect of various surfactants on TSST-1 production have shown that they can have a dramatic effect on production of this toxin.[71] These results suggest another tampon characteristic (i.e., type of surfactants present) that might influence the risk of menstrual TSS in users of various brands and styles.

The early case–control studies also examined the role of a number of other factors in determining the risk of developing TSS during menstruation. Four of the studies found that women with TSS were less likely to use oral contraceptives than controls, although the differences in individual studies were not statistically significant.[5,6,15,58] One study found that continuous use of tampons during the menstrual period was associated with an increased risk of TSS,[6] while in another study a similar association, present on univariate analysis, did not remain significant in a multivariate analysis.[15] Of four studies looking at the relationship between a history of a recent vaginal infection and the risk of TSS, only one found such an association. Factors found not to be related to the risk of developing menstrual TSS in one of more studies included marital status, income, parity, sexual activity, bathing, frequency of exercise, alcohol use, smoking, history of vaginal herpes infections, and frequency of changing tampons.

5.11.2. Postpartum TSS. Although numerous cases of TSS occurring during the postpartum interval have been reported, the lack of precise information concerning the incidence of TSS in various settings makes it difficult to be certain that the incidence in postpartum women is elevated. Those cases of postpartum TSS not related to infection of a

cesarean section incision or an infection of the breast (i.e., mastitis and breast abscess) have occurred predominantly in association with the use of tampons to control the flow of lochia or the use of barrier contraception (i.e., diaphragms and contraceptive sponges).[30]

5.11.3. Postoperative TSS. TSS has been associated with *S. aureus* surgical wound infections following a wide array of surgical procedures.[68] It has been suggested, however, that patients undergoing nasal surgery are at particularly high risk, presumably due to the frequency of *S. aureus* carriage in the nasopharynx and the difficulty of eradicating such carriage.[22] The common use of "nasal tampons" and other packing material following nasal surgery also may play a role.

5.11.4. Other Nonmenstrual TSS. TSS can result from *S. aureus* infection at any body site. However, many nonmenstrual TSS cases are the result of cutaneous and subcutaneous *S. aureus* infections. Also, cases of TSS associated with *S. aureus* infection of the respiratory tract in the setting of influenza have received substantial attention.[72] Risk factors for the development of such infections and/or associated TSS have not been studied. A relatively small proportion of nonmenstrual, nonpostpartum TSS cases are associated with vaginal *S. aureus* infections. One risk factor that has been identified in such cases is the use of contraceptive sponges.[62] It remains uncertain whether or not diaphragm use is similarly associated with an increased risk of nonmenstrual TSS.

6. Mechanisms and Routes of Transmission

Like all *S. aureus* strains, those capable of causing TSS appear to be transmitted readily by person-to-person spread, both within the hospital and in the community. There is convincing evidence that a nurse transmitted a TSS-associated strain of *S. aureus* to hospitalized burn patients[67] and suggestive evidence that some cases of postoperative TSS are due to nosocomial spread of the causative organism by hospital personnel.[68,69] In addition, vertical transmission from mother to newborn, with the development of TSS in both, has been reported.[73] Outside the hospital setting, transmission between husband and wife has been suggested by the almost simultaneous appearance of TSS in both, as has transmission between mother and daughter pairs (unpublished reports to the CDC). It is assumed, but not proven, that transmission in all these instances was by direct person-to-person spread. Nevertheless, it should be emphasized that in many TSS cases, particularly those associated with a focus of infection in the vagina, it may well be that the disease is due to the introduction and/or multiplication of an endogenous *S. aureus* strain rather than to an exogenous source of infection.

7. Pathogenesis and Immunity

TSS results from an infection with an appropriate strain of *S. aureus* in a susceptible host. Once a nidus of infection is established, onset of symptoms typically occurs 1–3 days later. The best evidence concerning incubation period comes from patients with postoperative TSS due to surgical wound infections. In these patients, the date when the infection became established is usually the day of surgery, and thus can be determined unequivocally. The median incubation period in such patients is 2 days.[68] When TSS is caused by *S. aureus* infection of the vagina during menstruation, onset of symptoms is typically on the third or fourth day of menstruation, although it can be earlier or later.

In most cases of TSS, the toxin TSST-1 is the bacterial product most likely to be responsible for many of the observed signs, symptoms, and abnormalities of laboratory values. There is, however, substantial evidence that one or more other staphylococcal products, particularly enterotoxin B, are capable of causing an indistinguishable illness.[44,46] Furthermore, bacterial products other than TSST-1 that are made more commonly by *S. aureus* strains recovered from patients with TSS than by other strains have been described (see Section 4). For these reasons, it is likely that other staphylococcal products play a role in the pathogenesis of TSS. Furthermore, there is evidence that some of the multisystem derangements frequently observed in patients with TSS are due to the profound hypotension or shock that can occur and only indirectly to any staphylococcal products. For example, renal failure in TSS is probably secondary to hypotension-induced acute tubular necrosis, which in turn is the result of multiple factors, including hypovolemia due to vomiting, diarrhea, increased insensible losses associated with a high fever, and inability to ingest or retain fluids; and "thirdspacing" of fluids. Uptake of "endogenous" endotoxin from gram-negative intestinal flora also has been proposed as playing a role in the pathogenesis of TSS.[74]

The biological properties of TSST-1 have been studied in vitro and in vivo, and attempts have been made to develop an animal model of TSS. In vitro, purified TSST-1 has been shown to stimulate the proliferation of T lymphocytes,[75] to inhibit immunoglobulin synthesis,[76] and to be a potent stimulator of interleukin-1 production by macrophages and monocytes.[77,78] TSST-1 also has been shown to bind to and be internalized by epithelial cells,[79] suggesting that it can be absorbed from focal sites of infection. In vivo, TSST-1 has been shown to be pyrogenic,[40] to induce lymphopenia,[80] to

decrease the clearance of endotoxin,[81] and to increase susceptibility to endotoxin-induced shock.[74] It is now clear that many of the effects of TSST-1 are due to its potent "superantigen" properties, acting through the release of immune cytochines.[82] While initially reported to be an enterotoxin (as evidenced by induction of vomiting in monkeys), preparations of TSST-1 not contaminated with other staphylococcal enterotoxins do not appear to induce vomiting.[83]

Attempts to reproduce TSS in animals have included using mice, rabbits, goats, baboons, chimpanzees, and rhesus monkeys.[55,84–93] In these studies, investigators either have attempted to infect the animals with TSS-associated *S. aureus* strains at one of a variety of sites (previously implanted subcutaneous chambers, vagina, uterus, and muscle) or have injected purified TSST-1 as a bolus or continuous infusion. The animal models that come closest to reproducing the syndrome observed in humans have been those using rabbits. Live TSS-associated *S. aureus* organisms inside a previously implanted subcutaneous chamber and continuous infusion of purified TSST-1 both result in fever, hyperemia of mucous membranes, hypocalcemia, elevated creatinine phosphokinase (CPK) and hepatic enzymes, renal failure, and death in a high proportion of rabbits.[84,88] Also, the pathological changes observed postmortem in these animals are similar to those reported in patients dying of TSS.[94,95]

These rabbit models have been used to study the host factors in susceptibility to TSS suggested as important by clinical and epidemiological data or by in vitro results. It has been found that the age, sex, hormonal status, and strain of rabbits used all have a substantial impact on susceptibility to "rabbit TSS."[85,86] Thus, older rabbits have been reported to be more susceptible than younger rabbits. Similarly, male rabbits appear to be more susceptible than female rabbits, although castration abolishes this difference and estrogens protect male rabbits. Experiments concerning the contribution of endogenous endotoxin (i.e., endotoxin released by gut flora) to the pathogenesis of TSS have yielded conflicting results, although it appears that blocking the effect of endotoxin by giving polymyxin B does not consistently prevent rabbit TSS.[88] Preexisting anti-TSST-1 antibody, however, does appear to protect against TSS in the rabbit,[91] and corticosteroids in high doses also decrease mortality.[88]

Because of the observed association between menstrual TSS and tampon use, many investigators have looked at the effect of tampons and their constituents on the growth of *S. aureus* and the production of TSST-1 in vitro. At the same time, the effect of environmental conditions such as pH, PO_2, PCO_2, and cation concentration on the production of TSST-1 has been investigated. In general, studies have shown that tampons and their individual components inhibit the growth of *S. aureus* in vitro, regardless of the growth medium.[96,97] Although some studies have suggested that *S. aureus* can use various tampon constituents as an energy source, these results have been challenged and their relevance to human disease questioned.[98–100] Also controversial is the effect of tampons on the production of TSST-1 in vitro, with some studies showing that certain tampons and tampon constituents increase TSST-1 production and other studies showing no effect or inhibition of toxin production.[97,99] It has been suggested that the effect of tampons on TSST-1 production (and possibly on the risk of menstrual TSS) is mediated by changes in the availability of magnesium, which is bound by certain tampon components.[101] The various types of surfactants found on tampons also appear to influence the production of TSST-1, at least in vitro.

Growth conditions appear to be important in determining the amount of TSST-1 produced. Thus, an aerobic environment, neutral pH, and low levels of glucose, magnesium, and tryptophan all increase TSST-1 production, although some controversy has arisen about the effect of magnesium concentration on TSST-1 levels.[101–105] It has been shown that in patients with TSS related to focal sites of *S. aureus* infection, growth conditions within the infected focus are well suited to TSST-1 production.[105] Also, while the vagina generally has been considered to be anaerobic, studies have shown that a substantial amount of oxygen is introduced when a tampon is inserted, leading to speculation that the amount of oxygen introduced with a tampon may be an important factor in explaining the increased risk of menstrual TSS among tampon users.[106] A role for proteases of either bacterial or human origin in the pathogenesis of TSS also has been suggested.[105] An earlier theory that the association between menstrual TSS and tampon use was mediated by the demonstrated induction of vaginal ulcerations by tampons[107] has received less attention ever since similar vaginal ulcerations were reported in at least one patient with menstrual TSS who did not use tampons.[94]

8. Patterns of Host Response

8.1. Clinical Features

An illness meeting all the criteria of the established TSS case definition is, by the very nature of the criteria, severe, and the majority of such patients are hospitalized for treatment. Some patients experience the relatively gradual onset of sore throat, fever, fatigue, headache, and myalgias over 24–48 h, followed by vomiting and/or diarrhea, signs of hypotension, and the appearance of the characteristic diffuse "sunburnline" macular skin rash. Other patients appear to

have a much more dramatic onset over the course of several hours, with some reporting that they can remember the exact moment when they suddenly felt overwhelmingly ill.

Because an established set of strict criteria is used to define someone as having or not having TSS, all of the cases so defined are, not surprisingly, alike, regardless of the site of infection with *S. aureus*. There is, however, some variation. The temperature elevation in patients with TSS, while sometimes modest, can be extreme, with temperatures in the range of 104–106°F being fairly common. The evidence of hypotension in an individual case can range from mild orthostatic dizziness to profound shock. The characteristic macular skin rash can be dramatic and obvious, with the patient appearing bright red throughout; it can be subtle and difficult to appreciate, particularly in dark-skinned individuals; or it can be localized. Similarly, the desquamation that occurs during convalescence (usually 5–15 days after the acute illness) can be of subtle flaking and peeling of skin on the face and/or trunk or can involve the loss of full-thickness sheets of skin, particularly on the fingers, hands, and feet. Depending on which systems are affected most prominently in an individual case, the multisystem involvement in TSS can produce rather different clinical pictures. In some patients the involvement of the mucous membranes (e.g., sore throat, conjunctival and oropharyngeal injection) is severe and most prominent, while in other patients the gastrointestinal symptoms (vomiting and/or diarrhea) are predominant. Similarly, myalgias, thrombocytopenia, and involvement of the hepatic and renal systems can range from nil to severe. One study has suggested that the clinical spectrum of disease differs between menstrual and nonmenstrual TSS cases.[45]

Patients who receive aggressive supportive therapy (e.g., fluids), appropriate antimicrobial agents, and drainage of any focal *S. aureus* infection usually respond rapidly and improve over the course of several days. However, patients in whom therapy is either delayed or in whom a focal *S. aureus* infection is not eradicated can have a stormy, life-threatening course. In cases meeting all the established criteria, the case-fatality ratio is 1–3% overall, although it increases with increasing age.[19]

The spectrum of illness of TSS has not been defined adequately due to the lack of a specific diagnostic laboratory test. It is evident that some illnesses not meeting all the criteria of the strict case definition, which was devised for use in epidemiological studies, represent milder forms of TSS. For example, few would question that an individual whose highest recorded temperature was 101.8°F, but who otherwise met all of the established criteria, had TSS. A number of authors have described patients of this kind,[108,109] and some have attempted to fashion simplified and/or less rigorous case definitions for TSS.[110] It is apparent that less rigorous case definitions are likely to be more sensitive but less specific in identifying TSS cases. Ultimately, however, it is not possible, in the absence of a specific diagnostic test, to determine where along a spectrum of increasingly milder and/or more atypical cases illnesses cease to be TSS and start to be something else. Thus, it is unclear whether a tampon-using menstruating woman with *S. aureus* in the vagina (or anyone else) who experiences headache, fatigue, and nausea could represent a very mild form of TSS. Such distinctions are made all the more difficult because of the relative frequency with which completely asymptomatic individuals are colonized with TSST-1-producing *S. aureus* in the nasopharynx, vagina, and probably other sites that are not normally sterile.

8.2. Diagnosis

As noted above, TSS can occur in individuals of any age, sex, and race. However, most recognized cases occur in a limited number of clinical settings. In women of reproductive age, TSS is most commonly seen during the menstrual period and the postpartum interval, although it can occur at other times as well, in association with focal *S. aureus* infections and in users of barrier contraception. TSS during pregnancy, however, is quite uncommon. Although patients undergoing nasal surgery may be at elevated risk, TSS related to a surgical wound infection is a possibility in any postoperative patient, particularly during the first 24–72 h. In many such instances, there will be few or no local signs that the operative site is infected.[68] As noted above, the median interval between surgery and onset of TSS in such cases is 2 days, but the range is 12 h to many weeks. TSS in an infrequent but serious consequence of focal *S. aureus* infections at every conceivable body site, although cutaneous and subcutaneous abscesses and other similar infections appear to predominate. In addition, TSS has been reported to be a life-threatening complication of postinfluenza *S. aureus* infections of the respiratory tract.[111,112]

The differential diagnosis for a patient suspected of having TSS depends, in part, on which features of the illness are most prominent. For example, patients in whom sore throat and fever predominate early are frequently suspected initially of having streptococcal or viral pharyngitis. In cases in which diarrhea and vomiting are more prominent, viral gastroenteritis is often considered. When the rash becomes apparent, scarlet fever, streptococcal TSS, and drug reactions are often suspected.

The differential diagnosis also can be influenced by the patient's age and sex and the clinical setting in which the illness occurs. For example, cases in infants and very young children must be distinguished from Kawasaki

Table 3. Differential Diagnosis in Patients with Suspected TSS

Kawasaki syndrome
Scarlet fever
Meningococcemia
Leptospirosis
Measles (especially "atypical")
Rocky Mountain spotted fever
Viral gastroenteritis
Viral syndromes with exanthems
Appendicitis
Pelvic inflammatory disease
Tubo-ovarian abscess
Staphylococcal scalded skin syndrome
Drug reactions/Stevens—Johnson syndrome

syndrome and staphylococcal scalded skin syndrome. Similarly, in postpartum or postabortion women, other causes of fever and hypotension must be considered, such as endometritis and septic abortion. In individuals with appropriate exposure histories, leptospirosis, measles, and Rocky Mountain spotted fever should be included in the differential diagnosis. In summary, TSS can be confused fairly readily with a wide range of other conditions (Table 3).

9. Control and Prevention

9.1. General Concepts

9.1.1. Menstrual TSS. Most strategies for decreasing the incidence of TSS have focused on menstrual TSS and its relationship to tampon use. In light of the demonstrated association between tampon use and risk of developing menstrual TSS, women were advised in 1980 that they could minimize their risk of developing menstrual TSS, women were advised in 1980 that they could minimize their risk of developing menstrual TSS by not using tampons. In response, many women stopped using tampons, at least temporarily. The proportion of menstruating women who used tampons fell from approximately 70% in 1980 to less than 50% in 1981, but has rebounded to approximately 60–65% since that time.

In response to epidemiological and in vitro laboratory evidence concerning the possible roles of tampon absorbency and chemical composition in determining risk of menstrual TSS, most tampon manufacturers have dramatically altered both the absorbency and chemical composition of their products. After increasing markedly in the late 1970s, the measured in vitro absorbency of tampons has dropped sharply since 1979–1980, and one component, polyacrylate, has been eliminated from tampon formulations. In addition, one brand of tampons found to be associated with a high risk of menstrual TSS was withdrawn from the market altogether in 1980. All tampons sold in the United States 2005 were made of cotton, rayon, or a blend of cotton and rayon.

All tampons now carry a label explaining the association between tampon use and menstrual TSS and describing the signs and symptoms of the illness. Tampon packages also carry a statement that women should use the lowest absorbency tampon consistent with their needs. Uniform absorbency labeling of tampons was required by the Food and Drug Administration beginning in 1989.

Although frequent changing of tampons has been recommended as a way of decreasing the risk of menstrual TSS, there is no evidence to suggest that changing tampons more often reduces risk. Evidence from one study suggests that alternating tampons and napkins during a menstrual cycle may decrease the risk of TSS.[6]

9.1.2. Postpartum TSS. Because women may be at increased risk of TSS during the postpartum period, they should avoid the use of tampons and barrier contraception during that interval.

9.1.3. Hospital-Acquired TSS. Other than those measures designed to minimize nosocomial infections in general (e.g., good hand-washing practices) and those recommended specifically for patients with other types of staphylococcal infections, there are no proven methods for decreasing the risk of TSS associated with infected surgical wounds and other nosocomial *S. aureus* infections.

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis

Appropriate antimicrobial therapy of an initial episode of menstrual TSS, combined with discontinuing tampon use, has been shown to reduce the risk of recurrent episodes during subsequent menstrual periods.[5] The value of follow-up cultures and prophylactic antimicrobial agents in women with a history of menstrual TSS is unproven, although such measures may be justified in women who have had recurrent episodes of TSS. Because carriage of *S. aureus* at various body sites is so common and cases of TSS are relatively rare, there is no role for obtaining cultures from or giving chemoprophylaxis to individuals without a prior history of TSS.

9.3. Immunization

Although some consideration was given to attempting to develop a toxoid vaccine from TSST-1 soon after its discovery, no concrete steps in this direction have been taken. Given the high proportion of the population with naturally occurring anti-TSST-1 antibodies and the relative rarity of

TSS, it would be prohibitively expensive and impractical to demonstrate that such a vaccine yielded clinical protection.

10. Unresolved Problems

Unresolved problems in our understanding of TSS relate primarily to its pathophysiology. While a clear link between the use and risk of menstrual TSS has been established, the specific characteristics of tampons responsible for this increased risk are unknown. The relative importance of absorbency, chemical composition, oxygen content, and perhaps other tampon characteristics, such as the surfactants used in their manufacture, in determining risk is uncertain. Similarly, while a direct correlation between measured tampon absorbency and risk of menstrual TSS has been demonstrated, it remains unclear whether or not users of the lowest absorbency tampons are at greater risk than nontampon users. At the same time, the role of tampon chemical composition in determining risk is ill-defined. As a result of all these uncertainties, it is unknown whether or not the "perfect tampon" (i.e., one that offers menstrual protection and has no associated increased risk of menstrual TSS) currently exists or can be developed.

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CHAPTER 40

Tuberculosis

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1. Introduction

Tuberculosis is a contagious disease spread through the air in tiny droplet nuclei. Propelled into the air by the cough, sneeze, or speech of a person sick with pulmonary tuberculosis, *Mycobacterium tuberculosis* bacilli can stay suspended for prolonged periods of time, ready to be inhaled by others sharing the same airspace. *M. tuberculosis* usually results in latent infection controlled by cellular immunity and may later progress to active disease. Though primarily a pulmonary disease, tuberculosis can affect many organs, and continues to be a major cause of illness, disability, and premature death throughout the world. Tuberculosis has been curable for more than 50 years; a standard drug regimen of specific antibiotics for a minimum of 6 months is required.

In 1997 the World Health Organization carried out the first worldwide survey of the global burden of tuberculosis and estimated that on average, one person is infected with tuberculosis every second of every day.[1] In fact, one-third of the world's population, approximately 2 billion people, is infected with TB. Those that are infected are often concentrated in populations that suffer from crowding, poor nutrition, and all the other manifestations of poverty. Even so, most of these people will probably never know they are infected and will never develop active TB disease.

The epidemiology of tuberculosis has changed dramatically over the last decade in some parts of the world; human immunodeficiency virus (HIV) infection has struck a punishing blow to even well-functioning TB control programs. The impact is so massive that, despite improvements in case-finding and treatment completion, tuberculosis increased at an annual rate of 1% worldwide, until it peaked and began to stabilize in 2005.[2] The widespread development and subsequent transmission of multidrug-resistant tuberculosis in regions suffering from collapsing public health infrastructure has added yet another dimension to the difficulties in controlling this ancient human disease.

In 1998, the complete genome of *M. tuberculosis* was fully sequenced. The genome comprises 4,411,529 base pairs and contains around 4,000 genes. *M. tuberculosis* differs radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis and to two new families of glycine-rich proteins.[3] The sequencing of the genome has resulted in numerous advances in TB epidemiology, pathogenesis, diagnostics, drug development, and vaccines.

2. Historical Background

Descriptions of tuberculosis can be recognized in many older medical writings, usually as *phthisis* or *consumption*, described as a chronic wasting illness associated with *ulcerations* of the lungs; as *galloping consumption*, the pneumonic form of the disease; or as *scrofula*, an involvement of the cervical lymph nodes. That the various forms of tuberculosis had a common and infectious etiology was first established by Villemin in 1865. He showed that inoculation of material from the various forms of tuberculosis lesions caused a similar disease in rabbits, as did the inoculation of sputum from patients with pulmonary lesions. Klebs also demonstrated the transmissibility of tuberculosis from man to animals in 1877, and even produced culture media for the growth of tubercle bacilli.[4]

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Five years later, in 1882, Robert Koch announced his discovery of the tubercle bacillus and a means of staining it for microscopic examination. Only then did it become feasible to make a definitive diagnosis of tuberculosis and to identify disseminators of the organism. Koch made another fundamental contribution to the epidemiological armamentarium by producing tuberculin in 1890. Although his claim for the therapeutic use of tuberculin was discredited, it was soon recognized that an infected person could be identified by the local reaction that followed the injection of a small amount of tuberculin into the skin. The third major diagnostic procedure resulted from the discovery of X-rays by Roentgen in 1895. Radiographs of the chest made it possible to demonstrate pulmonary disease before it could be recognized clinically and sometimes before it became communicable.

Refinements of these fundamental diagnostic procedures occurred for the most part during the 1940s and 1950s and added greatly to their usefulness. Development of specific culture media made it possible to detect much smaller numbers of tubercle bacilli than could be demonstrated by microscopic examination of stained specimens; furthermore, cultures were less expensive and more widely applicable than guinea pig inoculations, which were originally used to confirm microscopic examinations. Photofluorography brought chest radiography to the general population in mass surveys designed to detect early asymptomatic cases of pulmonary tuberculosis. Improvements in the standardization, administration, and interpretation of the tuberculin test made it possible to identify, albeit with some misclassification, people who had been infected with *M. tuberculosis*.

In recent years, new molecular techniques, such as nucleic amplification techniques (e.g., polymerase chain reaction), now make it possible to detect even smaller numbers of tubercle bacilli in biological specimens in as short a time as several hours, to rapidly identify organisms in culture.[5] Furthermore, DNA fingerprinting techniques, such as restriction fragment length polymorphism analysis and spoligotyping, allow the transmission of tuberculosis to be elucidated, adding significantly to epidemiologic research and public health practice.

From the 1860s to the 1940s, tuberculosis killed more people each year in the United States than any other contagious disease—one out of every seven deaths was from TB. In 1861 Oliver Wendell Holmes referred to consumption as the *white plague*. The history of tuberculosis control, in Europe and the United States, is closely entwined with the sanatorium movement, which espoused healthful living, with particular emphasis on rest, good food, and fresh air. In addition, the isolation of patients in sanatoria during their "treatment" undoubtedly reduced the risk of transmission of their disease to others. Historian JoAnne Brown found that following the civil war, military metaphors proliferated, many of them harkening back to the days of knighthood, an era from which civil war soldiers took inspiration, espousing chivalric deeds. It was in this social environment of a sanctified "battle" against germs that the Lorraine cross was selected as the symbol of the efforts to combat lung diseases, including tuberculosis.[6]

Although the basic precepts of the sanatorium movement can be traced back to the temples of Asclepius, (or Aesculapius, as he was known to the Romans), they did not receive wide acceptance as treatment for tuberculosis until the successful establishment of sanatoria by Brehmer in the mountains of Silesia in 1854 and by Trudeau in the Adirondack mountains of New York in 1882. Sanatorium treatment became the major tuberculosis control activity for decades, diminishing in importance only with the introduction of effective antibiotics, notably streptomycin in 1946, para-amino salicylate (PAS) in 1948, and isoniazid in 1952. Successful treatment on an outpatient basis then became possible, and the days of the sanatoria came to an end over the ensuing decade. The demonstration in India in the early 1960s that ambulatory therapy was safe and effective for both patients and their family contacts made hospitalization or institutionalization of TB patients unnecessary and solidified the emphasis on outpatient treatment. Further advances in the treatment of tuberculosis and discovery of other antimycobacterial drugs have led to shortening of therapy to 6 months and intermittent administration of medications.

Specific preventive measures began with the development of an attenuated strain of *Mycobacterium bovis*, a member of the MTB complex, by Calmette and Guérin between 1908 and 1919. The vaccine prepared from this strain came to be called bacille Calmette–Guérin, or BCG. Accepted at first with reluctance, subsequent clinical trials demonstrated substantial protection from TB and death, and use of the vaccine increased over several decades. BCG is a live attenuated vaccine, and no seed lot of the original strain has been preserved. As discussed below, multiple progeny of the original BCG are now in use globally, and vaccination with one of the many substrains has become a mainstay of childhood immunization programs throughout the world.

During the 1950s, large-scale controlled trials demonstrated that people infected with tubercle bacilli could be protected from the subsequent development of disease by the administration of isoniazid. Subsequent studies have demonstrated the risks and benefits of isoniazid (and rifamycin) for treatment of latent TB infection, but use of preventive therapy remains curiously limited in most of the world.

Tuberculosis mortality began to decline in developed countries prior to the present century, long before

tuberculosis control measures could have had any general effect. Dubos and Dubos[4] pointed out that where the decline could be documented by death records, sanitary and social reform movements in the early nineteenth century brought about many improvements in living conditions that would tend to operate against the tubercle bacillus. At the same time, in parts of the developing world, through colonization and the changing of political boundaries, tuberculosis was being introduced to naïve populations with devastating effects. More specific tuberculosis control measures, such as the use of drugs, played a major role in maintaining the decline throughout most of the world, especially during the later decades of the twentieth century.

Following the introduction of effective drug regimens, the public health and medical communities believed that tuberculosis was a problem of the past and interest waned. Tuberculosis control and research budgets began to be cut, and preventive measures were curtailed. Meanwhile, conditions favoring tuberculosis did not diminish. The divide between the rich and poor continued to become more extreme; refugee populations (including those internally displaced) are in the tens of millions; poverty and all its accompanying attributes of crowded living conditions, poor nutrition, and diminished access to appropriate health care continue to provide conditions ideal for the spread of this infection.

In the early 1980s, when the human immunodeficiency virus (HIV) was being recognized, tuberculosis control efforts in the United States were at an all-time low. It became apparent early on that HIV drastically reduced resistance to tuberculosis. At the beginning of the 1990s, multidrug-resistant tuberculosis became a major problem, particularly in New York City and surrounding areas, fueled by collapsing health systems and HIV infection. The concern engendered by the emergence of strains of tuberculosis that were potentially incurable resulted in an increase in interest and funding.

The World Health Organization (WHO) has played a leadership role in bringing tuberculosis control back to the forefront as a global priority. The Bill and Melinda Gates Foundation has identified tuberculosis as an important disease to control and as a result has funded unprecedented research on improved diagnostics, drugs, vaccines, and public health strategies. The United States has set an ambitious goal of reducing TB incidence to less than 1 per million by 2010,[7] and the Group of Eight (G8) leading industrial economies has set the audacious goal of reducing TB prevalence and mortality to half the 1990 levels by 2015. The United States represents only one-half of 1% of all the tuberculosis in the world, so the battle will not be won or lost within its borders. Whether tuberculosis will be eliminated or not is open to question.

3. Methodology

3.1. Sources of Mortality Data

Worldwide tuberculosis deaths and death rates are tabulated in the *World Health Statistics Annual*, published by the WHO in Geneva, Switzerland. Deaths and death rates specific to the United States and its territories can be found in various national vital statistics publications, such as *Vital Statistics of the United States*, published annually by the National Center for Health Statistics, Centers for Disease Control and Prevention (CDC), US Department of Health and Human Services. Annual reports on tuberculosis are also published by the Division of Tuberculosis Elimination, CDC.[8] Useful compendia of older statistics are also available.[9,10]

Although mortality rates have been useful historically as indicators of tuberculosis trends and of regions where tuberculosis cases are concentrated, today, with the advent of effective chemotherapy, mortality rates are often better indices of the lack of access to good medical regimens or lack of adherence to them. Even this use may be jeopardized by the estimate that tuberculosis deaths are markedly underreported in much of the world.[11,12]

Autopsy studies may reveal tuberculosis deaths that would not have been captured by routine surveillance strategies. No longer done routinely, autopsy studies carried out in general or specific populations often reveal evidence of previously undetected active tuberculosis.[13,14]

3.2. Sources of Morbidity Data

Tuberculosis cases and case rates are tabulated and summarized in the publications from the WHO and CDC mentioned in Section 3.1. In addition, the *Morbidity and Mortality Weekly Report* from the CDC and monthly reports from state health departments give up-to-date accounts of reported domestic cases in the United States. However, in 2006 tuberculosis was no longer reported in MMWR, presumably because there were so few cases. The accuracy of TB case-finding and reporting is highly variable in developing countries.[15]

A distinction should be made between reporting TB cases and suspects to a health department and the counting of TB cases for the purposes of determining incidence of disease. Tuberculosis disease can be defined clinically and/or through evidence obtained through laboratory evaluation(s) of clinical specimens. Without laboratory evidence of *M. tuberculosis* complex or demonstration of acid-fast bacilli (AFB) in a clinical specimen, clinical case definitions require three criteria to be counted: evidence of TB infection based

on a positive tuberculin skin test result; signs and symptoms compatible with current TB disease or clinical evidence of current disease; and current treatment with two or more anti-TB medications.[16]

3.3. Surveys

Surveys for tuberculosis have been conducted by means of physical examinations, bacteriological examinations of sputum, chest photofluorography, and tuberculin testing. In 1917, an extensive antituberculosis campaign was carried out in Framingham, Massachusetts, in the course of which approximately 11,000 people, two-thirds of the population, were given physical examinations for tuberculosis.[17] The findings of this survey resulted in the estimate that there were nine prevalent active cases for every annual tuberculosis death, a ratio used for the next three decades in the United States to estimate the size of the tuberculosis problem.

Sputum surveys are limited as a general rule to people with abnormal findings on chest X-ray surveys either to identify cases that are most meaningful from the public health point of view or as a means of surveillance for drug-resistant organisms. Occasionally, in areas where mass radiography is impractical, sputum surveys of general populations have been done to estimate the prevalence of tuberculosis or to detect tuberculosis cases among people with chronic cough.

With the demonstration in Brazil by de Abreu and de Paula[18] in 1936 of the practicability of mass photofluorography, it became feasible to examine the lungs of large numbers of apparently normal people for tuberculosis. In the decade following World War II, millions of people were examined in mass chest X-ray surveys. While it seems reasonable to believe that detection of unrecognized cases of tuberculosis was beneficial, there is little evidence to substantiate this belief. In Wales, an intensive tuberculosis case-finding and treatment program was followed by a decreased risk of new infections among children,[19] and in Alaska, an intensive case-finding and treatment program was followed by a marked decrease in childhood infections.[20]

Use of the tuberculin skin test can contribute a great deal to epidemiological knowledge. The applications of tuberculin testing to the delineation of the risks of having been infected with tubercle bacilli by time, place, and person are epitomized by the work of Palmer and Edwards and their colleagues.[9,21–26] By careful attention to the selection of study populations, to the techniques of administering and reading tuberculin tests, and to the interpretation of frequency distributions of reaction sizes, it has been possible to learn a great deal about the prevalence of infected people under varied circumstances and the implications of these findings for temporal, geographic, and personal differences in the risk of becoming infected.

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organism. Tubercle bacilli are commonly looked for in sputum and other specimens by microscopic examination. Preparations stained by the Ziehl–Neelson acid-fast technique allow for the identification of the rod-shaped 1–4 μm long fuchsin-stained mycobacteria. Microscopic examination of stained specimens is relatively quick and inexpensive, but its sensitivity is low. It has been estimated that at least 5,000–10,000 bacilli/ml are needed to make their recognition by microscopy reasonably certain.[27] This is an important examination however; those patients with enough acid-fast bacilli (AFB) organisms to be visualized with a microscope, so called smear positive, represent patients who are the most infectious. With proper laboratory techniques in areas where tuberculosis is common, specificity is fairly high, since acid-fast bacilli seen on microscopy are usually tubercle bacilli; nontuberculous mycobacteria, however, have a similar appearance and can occur as pathogens, as normal inhabitants in oral or genitourinary secretions, or even in water used to wash the slides. However, in areas of high TB prevalence, the positive predictive value of AFB staining is quite high.

Culturing *M. tuberculosis* can detect as many as 30–50% more cases of active tuberculosis, making it much more sensitive than microscopic examination. The ability to visualize the AFB colonies growing on egg-based (Lowenstein–Jensen) and agar-based (Middlebrook agar) media also enhances the specificity. However, with a generation time of nearly 24 h, tubercle bacilli grow slowly, usually requiring 3–8 weeks to form identifiable colonies. Presumptive identification of the various mycobacteria is based on growth rate, colony characteristics, and pigment formation.[28] Faster culture systems that detect mycobacterial growth by sensing radioactive carbon liberation (BACTEC)[29] or oxygen consumption can identify growth in 1–2 weeks. The Mycobacteria Growth Indicator Tube (MGIT) system, a non-radiometric method, cultures specimens in an enriched broth contained in a glass tube. The tube also houses an oxygen sensor embedded in silicone at the bottom of the tube. As the *M. tuberculosis* consumes the oxygen, depleting it within the tube, the sensor becomes fluorescent, which can be easily observed with the use of an ultraviolet lamp.[27,30]

The use of nucleic acid probes or the ρ-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test can rapidly distinguish *M. tuberculosis* complex from nontuberculous

mycobacteria with considerable accuracy.[5] Nucleic acid amplification techniques have been approved for diagnosing TB in many countries. Both reverse transcriptase-polymerase chain reaction (RT-PCR) techniques and transcription-mediated amplification (TMA) assays can detect *M. tuberculosis* in sputum and other specimens within several hours. Despite the intuitive appeal of these molecularly based, rapid diagnostic assays, their use has been hampered by limited sensitivity and less-than-desirable specificity. Inhibitors present in sputum impede DNA amplification, reducing sensitivity to 85–90% in AFB smear-positive, culture-positive specimens and to only 50–70% in smear-negative, culture-positive specimens.

3.4.2. Serological and Immunologic Diagnostic Methods. The most widely used and thoroughly validated immunologic test for *M. tuberculosis* infection for epidemiological and clinical purposes is the tuberculin skin test. The standard tuberculin test is administered by the Mantoux technique, whereby 0.1 ml of a purified derivative of tuberculin is injected just under the skin of the forearm by needle and syringe. The definition of a positive reaction is best derived from a frequency distribution of reaction sizes in the population under study.[31–33] The optimal dividing line between positive and negative will vary with the proportion of people in the population who have been infected with tubercle bacilli, the frequency of cross-reactions caused by infections with BCG or nontuberculous mycobacteria, and technical factors related to administration and reading of the test.

Some account of these factors has been taken in the several definitions of a positive tuberculin reactor for use in the United States. If the likelihood of tuberculous infection is increased, as it is among people with abnormal chest radiographs or with a history of recent exposure to an infectious case, or in immunocompromised patients (e.g., HIV infection), reactions of 5 mm or more in diameter to 5 tuberculin units of PPD are considered positive and indicate the presence of tuberculous infection. In individuals from settings with an increased risk of TB infection or with medical conditions increasing the probability of developing active TB (e.g., diabetes, renal failure), 10 mm is the cut point. In people at low risk of tuberculosis infection, 15 mm of induration is the recommended cut point.[34] The higher cut point reduces sensitivity but improves specificity.

Although the accuracy of the tuberculin test in detecting past or present infection with tubercle bacilli compares favorably with that of many medical screening tests, there are important sources of error. Errors in dosage, depth of injection, and measurement of reactions can result in misclassification. False-negative reactions may occur up to 8 weeks after infection, in seriously ill patients, during and shortly after viral infections and vaccinations (notably measles), and with natural and induced immunosuppression (e.g., Hodgkin's disease, HIV infection, corticosteroid therapy). Much more common, however, are false-positive reactions caused by infections with nontuberculous mycobacteria or vaccination with BCG.

Identifying recently infected people by means of repeated tuberculin tests of initially negative reactors is simple in theory but difficult in practice. Even with fastidious technique, the results of two tests can vary by 6 mm or more.[35] Consequently, only people whose reactions change from negative to positive and increase by at least 10 mm are considered to have had a true change in tuberculin sensitivity that may have been caused by a recent infection.[36] A further complication in the detection of newly infected people results from the "booster effect," analogous to the anamnestic response in serology. Many people, especially those over 50 years of age, may have had a gradual decline in tuberculin sensitivity to the point where an initial test causes no detectable induration. In some of these previously reactive people, the stimulus of the initial test causes a "boost" of their sensitivity back to its original level.[37] Boosting occurs within 1 week and appears to last for up to 2 years. If these people are given a second test 6 or 12 months later, their positive reactions at that time may have resulted from either the booster effect or a new infection.

To minimize the possibility of falsely attributing a boosted reaction to a new infection, people who are to be tested periodically and who have a low risk of becoming infected (i.e., those who are not household contacts) should have a second test given a week after the initial test if that initial test was negative. People still negative to the second test should then be included in the periodic testing program, with reasonable assurance that any subsequent change to a positive reaction (with an increase of at least 10 mm) will have resulted from a recent infection. People who are positive to the second test of the initial series should be classified as initially positive reactors.[38]

A variety of serological tests for the diagnosis of tuberculosis have been evaluated but have been of limited value due to poor predictive values. Assays that detect cellular immune responses to *M. tuberculosis* infection rather than humoral responses hold considerable promise. There are currently two commercial interferon-gamma (IFN-γ) assays: QuantiFERON®-TB Gold assay[39,40] and T SPOT-TB[41,42] test. Both tests measure the production of IFN-γ by CD4 T-cells in response to specific *M. tuberculosis* antigens. An advantage of both tests is that unlike the TST, they react to only a few species of nontuberculous mycobacteria. This makes it possible to differentiate people infected with *M. tuberculosis*, from those with a history of BCG

vaccination, for example. Like the TST, these tests cannot differentiate between latent infection and active TB disease. A prospective study, completed in Italy in 2005, compared the results of QuantiFERON®-TB Gold assay and the T SPOT-TB test in 393 people suspected of having active or latent TB, 381 of them with known positive TB skins tests. Results of the blood tests were similar to previous studies in the level of agreement with the skin test, but determined that the rates of indeterminate and positive results from each assay differed significantly. The study incidentally discovered that both assays resulted in more indeterminate test results in immunosuppressed patients.[43] There is a reasonably good correlation between the results from these assays and the TB skin test, although they may be measuring responses of different components of cell-mediated immunity, with the major discrepancies in results related to prior BCG vaccination.[44] More, well-designed prospective evaluations of these tests will be necessary to determine their usefulness in identifying individuals with TB infection.

4. Biological Characteristics of the Organism

There are currently 82 recognized species of mycobacteria; most of them ubiquitous in the environment and not pathogenic to humans. *M. tuberculosis* complex includes *M. bovis* and *M. africanum*. Mycobacteria other than *M. leprae* and *M. tuberculosis* complex are referred to as nontuberculous mycobacteria (NTM).

M. tuberculosis is a small, slender (1–4 μm long by 0.3–0.6 μm in diameter), and slightly curved, rod-shaped bacillus that reproduces slowly, approximately once every 24 h. An aerobe, the bacteria are highly adapted to survive within the inhospitable environment of a human alveolar macrophage—inside the very cells designed to attack foreign elements that enter the tiny air sacs of the human lung. Using sophisticated mechanisms to utilize the host's iron and oxygen to survive, *M. tuberculosis* is able to persist for years, frequently for the lifetime of the host.[45]

Tubercle bacilli are particularly resistant to drying, a characteristic attributed to a waxy, lipid-rich cell wall. The high lipid content makes the bacilli resistant to dyes routinely used to stain organisms in laboratories. However once stained, the mycobacteria resist decolorization with acidified organic solvents and are there therefore called *acid-fast bacilli* (AFB), a name synonymous with mycobacteria.

The bacilli are also resistant to most ordinary germicides, particularly in the presence of organic material such as sputum. They are susceptible to moist heat, being killed in approximately 30 min by exposure to temperatures of 60°C and in less than a minute when greater than 70°C.

Laboratory studies have demonstrated that ultraviolet light can disrupt the DNA of *M. tuberculosis*, killing the bacteria. Sunlight and artificial ultraviolet light both appear to be effective in killing these air-borne pathogens.[46] Studies are ongoing to determine effective and safe methods to take advantage of the organism's vulnerability to exposure to these wavelengths.[47,48]

5. Descriptive Epidemiology

The incidence and prevalence of tuberculosis infection and disease vary throughout the nations of the world. Risks for acquisition of TB infection and development of disease can be classified into two major categories: extrinsic and intrinsic. Extrinsic risks include environmental and/or social factors that increase the degree of exposure to TB. Intrinsic risks are factors determined by each individual's genetic makeup, nutritional status, and specific health status. Many extrinsic risks vary by community. Intrinsic risks are constant across populations.

Tuberculosis differs from most other bacterial diseases in a very important respect, namely that the immune response to the primary infection is often not sufficient to rid the body of the organism. As a consequence, an unknown but significant proportion of tuberculin reactors continue to harbor living organisms and at risk of reactivation for the rest of their lives. In epidemiological terms, this means that the incubation period of tuberculosis is highly variable, ranging from a few weeks to a lifetime. Those who pass through the highest-risk period shortly after infection still have a subsequent lifetime risk that may actually exceed the initial risk because of the cumulative effect of a low risk operating over many years.

In thinking epidemiologically about the development of tuberculosis in populations, it is important to look at each of the two major stages of its pathogenesis—the acquisition of infection and the subsequent development of significant disease. While all infectious diseases must go through these two stages, their separation is particularly important in tuberculosis because the known risk factors for infection are so different from the risk factors for the development of disease after infection. Despite the voluminous literature on tuberculosis, very little of the work has been done in such a way that these two risks can be disentangled.

5.1. Prevalence and Incidence

5.1.1. Infection. Information on the prevalence or incidence of tuberculous infections is rarely available on a community-wide basis. Estimating the prevalence of infection can be particularly difficult because the tuberculin skin

test cannot differentiate between the positive reactions that may occur in response to infection with *M. tuberculosis*, nontuberculous mycobacteria, and BCG vaccination. The frequency of cross-reactions from nontuberculous mycobacterial infections varies markedly by geography, being almost nonexistent in the Arctic and common in some tropical and subtropical areas. However, PPD-S, the most widely used tuberculin, elicits larger reactions in individuals with *M. tuberculosis* infection than in those who have been sensitized by BCG or nontuberculous mycobacteria. Larger reactions (>15 mm) are most likely to be due to infection with tubercle bacilli than other mycobacteria.

The longer one lives, the greater one's risk of being exposed to tuberculosis. As a consequence, the prevalence of tuberculous infections rises with age, tending to level off at about 40–50 years. The incidence of tuberculosis infection is usually estimated from the change in prevalence of tuberculin reactors by age rather than from repeated testing of the same individuals.[49,50] This strategy minimizes errors in classification caused by technical variations in testing and reading and also those caused by the effects on the second test of the stimulus from the first test, the so-called booster effect (see Section 3.4.2).[51] The annual risk of infection (ARI) is calculated from tuberculin skin test surveys of representative samples of non-BCG-vaccinated children. For example, if a sample of unvaccinated 6-year-olds had a prevalence of TB infection of 6%, the annual risk of infection would be 1%.[52]

The incidence of new TB infections appears to have been decreasing globally over the past half century, though there is enormous variation by region.[20] Currently, The ARI is highest in sub-Saharan Africa (1.5–3.5%) and Asia (1.0–2.0%). In comparison, the ARI in the Netherlands in 1985 was estimated at 0.012%.

ARI can be used to estimate the incidence of sputum smear-positive pulmonary TB. Based on data from countries where both ARI and the rates of smear positivity were known, for every 1% ARI, there are approximately 49 cases (95% confidence interval 39–59) of smear-positive TB per 100,000 population.[52] This estimation, however, is based on data from developed countries in an era of TB decline and may not be representative of TB in developing countries at present, especially those countries with large HIV epidemics.

Another useful function of large-scale TB skin test surveys is to determine the relative differences in risks of infection between different population groups. Studies carried out in the last decade in Canada, Nepal, and Taiwan have all demonstrated that aboriginal populations continue to suffer disproportionate risk of infection with tuberculosis.[51,53,54] In India, data from ARI surveys carried out between 2000 and 2002 in a variety of locations throughout the country showed great variability in the risks of infection between rural and urban populations ranging from 1.2 to 3.8%.[55]

On the basis of a variety of data, including tuberculin surveys, back calculation from reported case rates, and other information, the estimated global prevalence of TB infection is 2 billion, or one-third of the world's population. This widely quoted figure is clearly only an estimate, but it is probably not too far off the mark, though certainly imprecise.

5.1.2. Disease. The frequency of tuberculous disease depends first on the prevalence of infection and second on the ability of infected people to contain that infection. The prevalence of a disease is a function of its incidence and average duration.

In 2003, there are believed to be 15.4 million prevalent TB cases globally (245/100,000), of which 6.9 million (109/100,000) were smear positive. An estimated 1.7 million people (28/100,000) died from TB in 2003, including those co-infected with HIV (229,000).[56] In 2004, an estimated 264,000 TB patients co-infected with HIV died.[57] The risk of tuberculosis disease varies widely throughout the world, the result of wide differences in the risk of infection and co-morbidities contributing to individual risk of disease if infected.

5.2. Epidemic Behavior and Contagiousness

Tuberculosis can and sometimes does occur in sharply localized outbreaks. In nearly all such instances, these involve the exposure of previously uninfected people to an infectious individual in crowded quarters. Perhaps the most remarkable aspect of these outbreaks is their relative rarity, given the frequency with which uninfected individuals are exposed to infectious cases in homeless shelters, schoolrooms, and factories.[58–62] A possible explanation is that some tuberculous patients are more effective spreaders than others. Another factor that may play a role is that tuberculosis is usually not highly communicable.[63] A study published in 2000 that reviewed the outcomes of contact investigations reported to the CDC from July 1996 through June 1997 for 1,080 pulmonary sputum-smear-positive TB patients (the most infectious TB patients), identified a total of 6,225 close contacts, 43% in the household. Thirty-six percent of all the contacts were TB skin test positive, and only 21% of children younger than 6 years of age were positive.[64] In contrast, secondary attack rates among 5- to 9-year-old household associates of cases of measles and mumps have been reported to be 81 and 86%. Because the communicability of measles or mumps is measured in days, while that of tuberculosis is measured in weeks or months, the infectiousness of tuberculosis per day of exposure must *on average* be very low indeed.

There is good evidence that the dosage of infection is related to the risk of developing tuberculosis.[65,66] Both among nurses in the Prophit Survey in England and white and Indian children in western Canada, positive tuberculin reactors who had been most heavily exposed were more likely to develop tuberculosis than tuberculin reactors with less exposure.[67,68]

The infectiousness of tuberculosis patients appears to be rapidly reduced by adequate chemotherapy.[47,69] The most convincing study to prove this point came from a controlled trials in Madras, India.[70] Newly diagnosed cases of active tuberculosis were randomly allocated to home or sanatorium treatment. Both groups received the same chemotherapy. At the end of 5 years, there were slightly fewer cases among contacts of patients treated at home than among contacts of those isolated in hospital. This result strongly suggests that chemotherapy was as effective as hospitalization in reducing the risk connected with being a close contact of an infectious patient.

When tuberculosis outbreaks occur among people infected with HIV, the dynamics of transmission change considerably because of the telescoping of the period between infection and disease and the great increase in the risk of disease following infection. The explosive nature of such outbreaks is well illustrated by experiences with multidrug-resistant tuberculosis in several hospitals in the United States and in France in the 1980s and 90s[71-73], and in South Africa in 2006.[74]

5.3. Geographic Distribution

Rates of tuberculous infection, disease, and death are highly variable, depending on location (Figure 1). An estimated 8.9 million incident cases of tuberculosis occurred in 2003 and an estimated 1.7 million died of tuberculosis. While case rates have been stable or declining in five of the six WHO regions of the world, the rate is increasing in the African region, due to the high prevalence of HIV infection (Figure 2), and in eastern Europe in response to the collapse of the Soviet Union and the resultant deterioration of public health services. The increases in Africa and eastern Europe have contributed to a net global increase in TB of $\sim$ 1% a year (Figure 3).[2,12,56,57] The 22 high-burden countries account for approximately 80% of all estimated new TB cases each year. China and India alone account for 35%.

Geographic differences measured only by official morbidity statistics may represent not only true differences in the frequency of disease but also diligence in searching for cases,

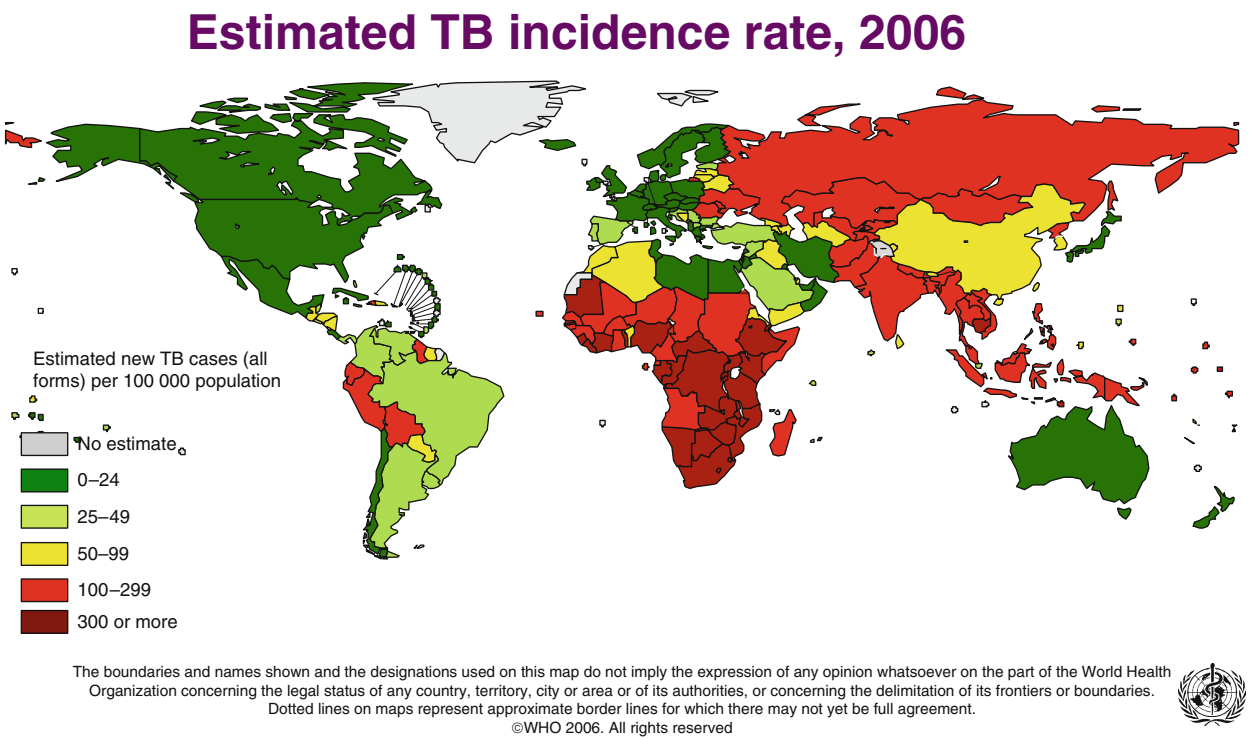

Estimated TB incidence rate, 2006
Estimated new TB cases (all forms) per 100 000 population
No estimate
0–24
25–49
50–99
100–299
300 or more
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.
Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.
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Figure 1. International tuberculosis notification rates in 2003.[56]
Global tuberculosis control: surveillance, planning, financing. WHO report 2008. Geneva, World Health Organization (WHO/HTM/TB/2008.393)

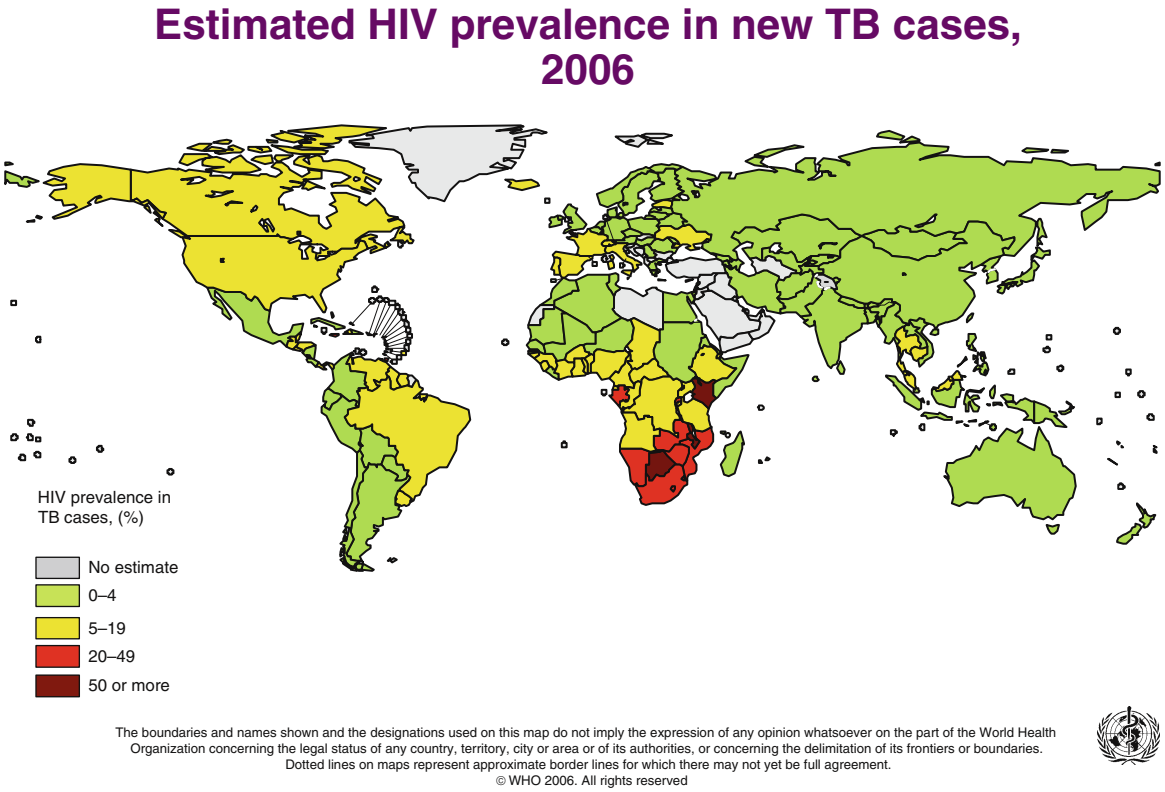

Estimated HIV prevalence in new TB cases, 2006
HIV prevalence in TB cases, (%)
No estimate
0–4
5–19
20–49
50 or more
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.
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Figure 2. Estimated numbers of HIV-infected tuberculosis cases per 100,000 population (all ages) by country in 2003.[56]
Global tuberculosis control: surveillance, planning, financing. WHO report 2008. Geneva, World Health Organization (WHO/HTM/TB/2008.393)

criteria for diagnosis, and perhaps completeness of reporting. Since its first global survey of tuberculosis in 1997, the WHO has improved our ability to make fair comparisons across the globe.[2]

Tuberculosis case rates for the United States are reported annually by the Centers for Disease Control and Prevention. In 2003, the highest case rates were found in District of Columbia, Hawaii, and California. Cases are more numerous in areas with many immigrants from high-prevalence counties, namely, southern California, areas along the Mexican border, much of Florida, and coastal areas in the northeast. More than 50% of the TB cases reported in the United States are now occurring among foreign-born individuals.[8]

Residence abroad has often been considered to be a significant risk factor for acquiring tuberculous infection. The risks of acquiring tuberculous infection when traveling are based on the same conditions that would apply had the traveler stayed home. The physical condition and vulnerability of the host and the length and extent of exposure would determine the risk.[75,76]

Travel by airplane has been shown to have its own special risks. Passengers sharing airspace for extended periods of time with an individual with active pulmonary TB can become infected within the time frame of the flight. The probability of infection is highest for those sitting closest to the effected individual.[77,78] However, it should be emphasized that transmission of TB on airplanes is extremely rare, and transmission in the community of household is responsible for the overwhelming majority of cases.

5.4. Temporal Distribution

The risk of disease following infection peaks in the first 1–2 years after acquiring infection then declines over time. In the large study of tuberculosis contacts conducted by the US Public Health Service, it was found that the attack rate among tuberculin-positive contacts was 1,000/100,000 during the first year after diagnosis of the index case.[79] Some 8–10 years later, their rate had fallen to 72/100,000 per year, not much higher than rates among tuberculin-positive individuals without a history of household exposure.

The lifetime risk of reactivation TB following latent infection is often estimated at 5–10%, but this depends on the age at which infection was acquired.[36] Data from children

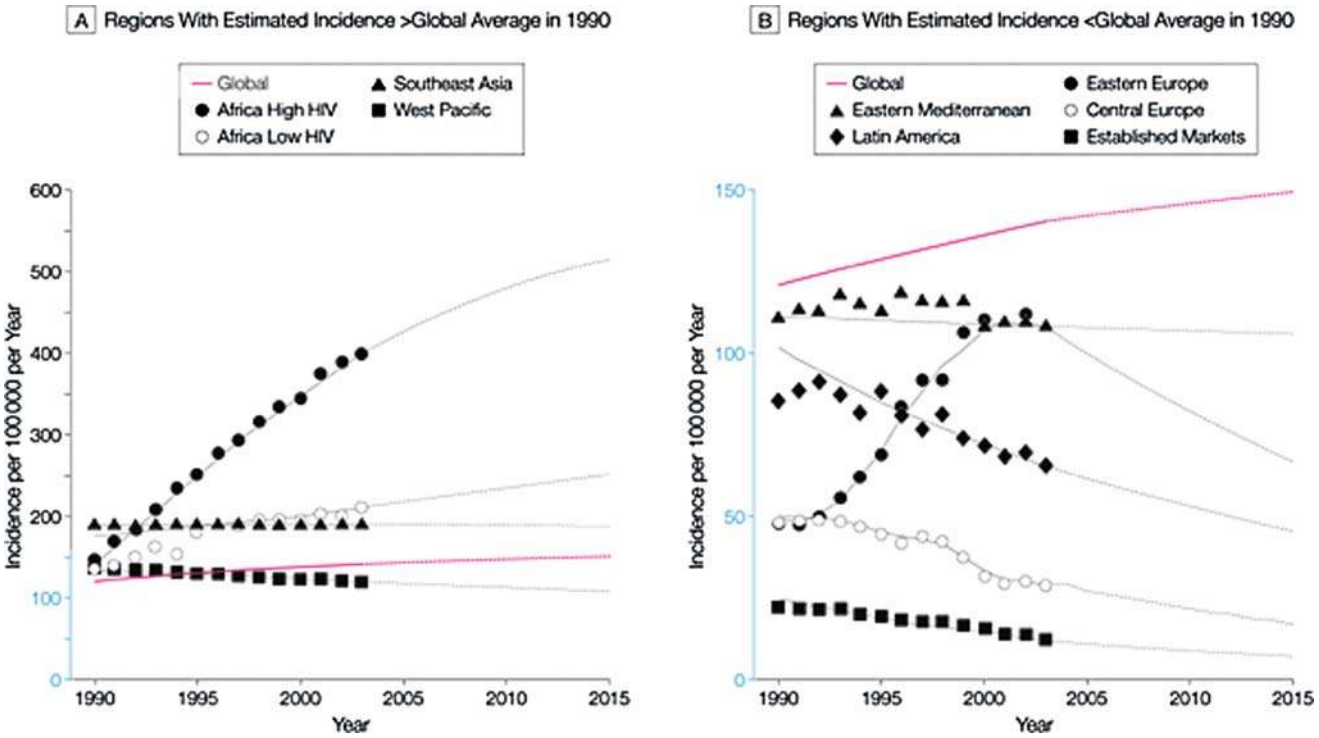


Figure 3. Trajectories of TB epidemic for nine epidemiologically different regions of the world.[2]

with positive tuberculin reactions who were followed for 19 years in Puerto Rico yields a lifetime risk for active TB of 10%, extrapolating linearly. This averaged risk substantially underestimates the risk for some while overestimating the risk for others. Risks vary according to age, co-morbidities, and size of the TB skin test reaction.[80]

5.5. Age

Tuberculosis mortality and morbidity rates show different patterns in populations with high and low risks of becoming infected with tubercle bacilli. Where the risk is high, there is a peak in infancy, followed by a second peak in young adult life, and sometimes a third peak in old age. Where the risk of becoming infected is low, tuberculosis case rates tend to increase steadily with age.[9]

The prevalence of positive tuberculin reactors usually increases at a fairly steady rate with age until about 40–50 years, when it tends to level off or even to fall somewhat. This was illustrated by results of tuberculin testing among New York City school employees in 1973–1974.[81] Determining the incidence of new infections with age is fraught with numerous problems. Most studies agree in finding an increase with age up to about 20 years; estimates of the incidence thereafter vary considerably.[50,82] When current risks of becoming infected are very low, it is likely that reversions from positive to negative may outnumber conversions, especially among older people.

The probability of developing tuberculous disease among positive tuberculin reactors also varies with age (Figure 4).[5,63,83] There is a peak in infancy and early childhood, followed by a second peak in young adult life. The pattern among people past the age of 45 is uncertain because studies of general populations have included only small numbers of infected people this old. It seems likely that the peak in infancy largely reflects the effect of a recent infection, since all infections among infants are necessarily recent. The reason for the increased incidence among infected people in young adult life is not known. The risk of developing tuberculosis at some point during a lifetime for an untreated infected infant is estimated to be approximately 10%. For adults past the age of 25 years, the lifetime risk is considerably lower, being influenced largely by the risk within a year or two after becoming infected.

5.6. Sex

In the United States, tuberculosis death and case rates have been lower among females than among males at almost all ages.[8,9] The differences between the sexes are slight

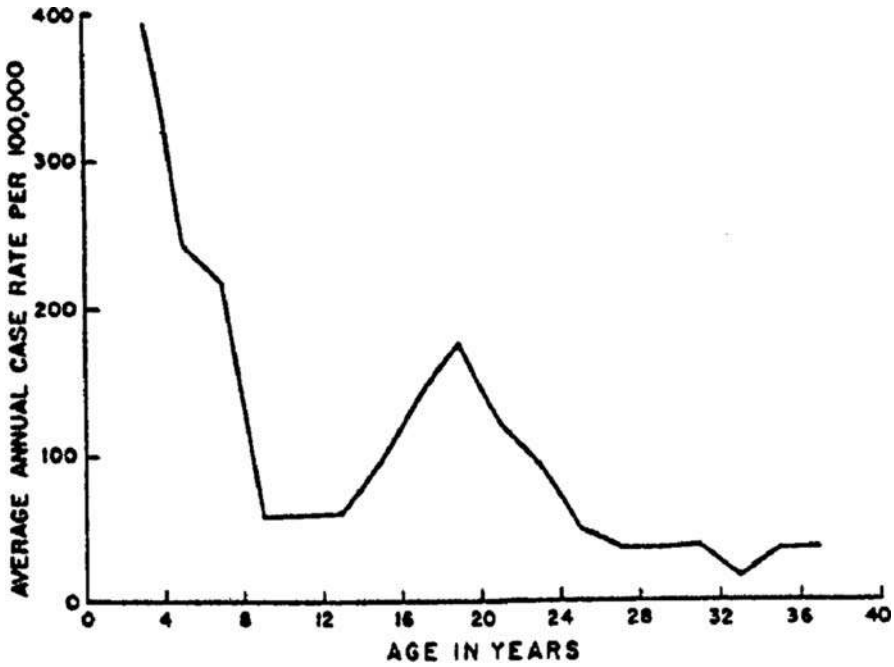


Figure 4. Incidence of tuberculosis disease among tuberculin reactors by age when tuberculosis was first diagnosed.[83]

until 25 years of age, following which male rates rise with increasing age to a greater extent than female rates. The prevalence of positive tuberculin reactors, and presumably the incidence of tuberculous infections, is also lower among females.[9,63]

It is difficult to determine whether the risks are inherent or environmental; and in some settings there are issues of access to care that suggest females with tuberculosis may have lower detection rates.[84,85] There is conflicting evidence regarding the risk of disease among infected males and females. Among Danes, the Alaskan Inuit, young blacks and Puerto Ricans in the United States and refugees in Indochina, rates were higher among females[63,86–88] whereas among whites in the United States and people in South India and Hong Kong, rates were higher among males.[89–91] In a naïve aboriginal population in Paraguay, where case rates were very high, a longitudinal study found no differences in rates of infection and/or disease between males and females.[92]

Most recently, a meta-analysis examined the male to female ratio of smear-positive TB notification rates in 28 high-burden countries. They determined that compared with countries that had a *per capita* cigarette consumption of ≤ 2 cigarettes/day, countries that had a *per capita* cigarette consumption of > 2 cigarettes/day were 78 times more likely to have TB notifications among males exceed those among females by $\geq 70\%$. While these results demonstrated a strong relationship between cigarette smoking and TB, they also provide support for the interpretation of sex differences in worldwide TB notification rates as indicative of true differences in the epidemiology of TB between males and females.[93]

5.7. Genetic Factors and Race

That varying degrees of resistance of tuberculosis can be inherited was clearly shown by Lurie in his work with inbred strains of rabbits.[94,95] The study of individuals with marked susceptibility to mycobacteria usually nonpathogenic to humans in addition to case–control studies using candidate genes and genome-wide screens of affected populations has provided substantial evidence for the role of genetic factors in the susceptibility to mycobacterial disease. Studies of rare mutations in IFN-γ and IL-12 signaling have established the importance of these components of immunity against mycobacterial infection in humans.[96,97]

It is probable that genetic factors are also important in keeping infection from progressing to disease. There have been numerous observations that the risk of tuberculosis among household contacts is greater for blood relatives than for others.[98] That there is a genetic component in this increased risk is indicated by studies of twins, in which concordance for tuberculosis is considerably greater among monozygotic twins than among dizygotic twins, even

when other factors related to tuberculosis risk are taken into account.[99] An additional indication of inherited susceptibility is the report of higher case rates associated with blood groups B and AB among the Inuit in Alaska.[100]

Differences in the presentation of disease also suggest genetic influence. In a study of the characteristics of officially reported tuberculosis cases, several striking differences were found. Alaskans (mostly Inuit and Indians) tended to have limited lesions that were rarely caseating. In contrast, patients from Singapore and from Maryland had extensive disease and a high frequency of cavitary lesions. Patients from five European centers tended to fall between these extremes.[101]

Historically, higher cases rates and deaths have been described among blacks, American Indians, and the Inuit than among whites in the United States.[9] A study of 150,000 nursing home residents in Arkansas in 1990 found that African Americans were twice as likely to develop TB as Caucasians, and this difference was not accounted for by any identifiable social or environmental factors.[102] Death rates have fallen somewhat more rapidly for nonwhites than for whites, while reported case rates have fallen somewhat more rapidly for whites.

Immigrants from developing countries are responsible for ever larger proportions of TB in the United States, Canada, Australia, and western Europe. Evaluating the risks of tuberculosis disease by ethnic group is generally carried out in countries experiencing massive immigration from the developing world.[9,103–107] Immigrants may not be fully representative of a particular ethnic group and the stresses related to their migration may influence their risks of developing TB disease.

5.8. Occupation

An analysis of proportionate mortality among 28 of the United States shows this index to be increased among low-level health care workers, funeral directors, people occupationally exposed to silica dust, and low-socioeconomic-status workers.[108] While mortality is an insensitive indicator of exposure risks, it does have the advantage of being available for all residents of many countries. For the more definitive risks of becoming infected or of developing disease, there is little available evidence with respect to occupation.[109]

Among health care workers, the measured risks vary between settings. In some hospital employees, there were only slight and nonsignificant differences in the frequency of positive tuberculin reactors by type of job or estimated exposure to patients after allowing for the fact that males, minority ethnic groups, and older people all had higher rates of positive reactions.[38] Studies done in New York City and another in the Samara Oblast of Russia evaluated the risk of latent TB infection in settings characterized as low and high risk. They found a significantly higher skin test conversion rate in occupational settings considered high risk over and above the background infection rates found in their respective populations at large.[110,111] Another study showed no increased risk of TB infection for health care workers; risk was explained by income and community, not occupation.[112] There is evidence that hospital physicians in training are not only at increased risk of becoming infected but tend to be uncooperative in tuberculin testing and preventive therapy programs.[113] In Turkey, it was student nurses who were found to be at particular risk.[113,114] The high rate among funeral directors was found to be related to the number of embalming procedures performed and the aerosols produced.[108]

Tuberculosis rates are often excessive in occupations with silica exposure.[115,116] Because silica has been shown to impair host resistance to tuberculosis in experimental animals, it is presumed that people with silicosis also have a reduced ability to control tuberculosis infections.[117] Evidence of the increased rates of tuberculosis among gold miners in South Africa, who are occupationally exposed to silica, appears to confirm that.[116,118–120]

5.9. Socioeconomic Factors

Tuberculosis has long been known to be associated with poverty, crowding, and malnutrition.[4,38] Mortality, morbidity, and prevalence of tuberculosis all tend to be highest in the poorest areas. In Washington County, Maryland, high-school children living in homes with one or more people per room or in an inadequate dwelling as assessed by the absence of complete bathroom facilities were more than twice as likely to be positive tuberculin reactors as children living in less crowded, more adequate dwellings.[121] A more recent study evaluated the association of poverty with TB incidence from 1984 to 1992 in New York City and determined that the relative risk of TB increased approximately 30% per 10% increase in poverty during that time frame.[122]

Relatively few studies indicate whether the risk of disease associated with socioeconomic status results from the risk of becoming infected, of becoming diseased after infection, or both. One of these studies, a report of tuberculin testing of New York City school employees in 1973–1974, showed that the frequency of tuberculous infection was indeed higher in personnel of lower socioeconomic status living in the poorest areas, 22.4%, contrasted with 5.5% in employees in areas with the highest socioeconomic rating, after adjustment for differences in composition by age, race, and sex.[63,81]

5.10. Smoking

In 1956, the British Medical Journal published two articles that discussed the relationship between smoking and pulmonary tuberculosis.[123,124] Doll and Hill made it clear that they had no doubts that there was an association between smoking and mortality from pulmonary tuberculosis, but that the habit of smoking was so strongly associated with social factors also intertwined with the risks of tuberculosis that it was not possible to determine that smoking was an independent risk.

The majority of research focusing on the effects of smoking on the risk of tuberculosis infection and disease has been carried out in recent years, and has been complicated by the likelihood that smoking may also affect delayed hypersensitivity to tuberculin, the very mechanism used to establish the presence of tuberculosis infection.[121,125] Research evaluating the relationship between smoking and tuberculosis disease and deaths has been more convincing. A prospective study carried out in Hong Kong demonstrated that in a cohort of 42,655 elderly people, followed for 2 years, a total of 286 active TB cases were identified. The annual TB notification rates were 735, 427, and 174 per 100,000 among current smokers, ex-smokers, and never-smokers, respectively. The trend in TB risk persisted after adjustment for other risk factors. Among current smokers, those who developed tuberculosis smoked more cigarettes per day than those who did not.[126] In an equally large study examining smoking and mortality in India, smoking was found to contribute substantially to tuberculosis mortality in men aged 25–69 years.[127] A meta-analysis carried out to determine the epidemiological basis for sex differences in TB notifications in 28 high-burden countries determined that cigarette consumption explained 33% of the variance in the sex ratio of TB notifications.[93] Although these studies may not have disentangled all the social factors related to smoking and the risks of tuberculosis, there are reasonable physiologic explanations, and the sheer magnitude of the morbidity and mortality attributed to this association should encourage closer attention to this modifiable risk factor that is associated with so many other health problems.[128]

5.11. Nutrition

For a long time, it has been observed that a variety of situations involving food deprivation are associated with increases in tuberculosis case and death rates.[4,9,21] Since the common factor in these situations is malnutrition, it is likely that the association is one of cause and effect. And since morbidity increases shortly after food shortages appear and decreases promptly when food supplies become adequate, it is likely that the effect of malnutrition is mediated primarily through a concomitant risk of disease among people already infected.

Studies on the importance of specific nutrients have been ongoing since at least the 1950s.[129,130] Epidemiological evidence suggests that vitamin-D deficiency, associated with deficient monocyte-macrophage function, may predispose toward vulnerability to tuberculosis.[131–133] Severe malnutrition has been shown to be associated with a diminution of tuberculin sensitivity following BCG vaccination.[134] There is no indication, however, that malnutrition protects against infection; rather, it appears that it diminishes delayed type hypersensitivity and causes false-negative skin tests.

Body build is related in part to nutrition. Studies among US Navy recruits demonstrated that there is no relationship of relative weight to the prevalence of positive reactions to tuberculin. However, among positive reactors on initial examination, subsequent case rates were nearly four times higher among men who were initially 10% or more underweight than among men initially 10% or more overweight.[26] That body fat was the component of weight likely to be responsible for this association was indicated by evidence from Muscogee County, Georgia. The incidence of disease was closely related to the thickness of subcutaneous fat measured from photofluorograms. Tuberculosis incidence was twice as high among people with less than 5 mm of subcutaneous fat as among those with 10 mm or more.[63]

Two recent placebo-controlled trials looked at whether multi-vitamins, zinc, and/or micronutrients improved treatment outcomes when administered with TB medications.[135,136] There were no dramatic improvements demonstrated in either study. Micronutrients provided a benefit of increased weight gain during treatment, and multivitamins in combination with zinc afforded a survival benefit in those TB patients co-infected with HIV. Both studies were carried out in Mwanza, Tanzania, in TB populations with high HIV co-infection (43%), making it difficult to generalize the findings, despite the elegant study designs.[135,136]

The studies discussed above do not conclusively answer the questions about nutritional status and tuberculosis. Although it is possible that undernutrition causes both leanness and susceptibility to tuberculosis, it is also possible that some other aspect of body build, such as hormonal or genetic factors, is the underlying cause of both. Two findings that favor the latter hypothesis are the impression that very few people in Muscogee County in 1946 could have been considered to be seriously undernourished and the observation among Navy recruits that tuberculosis contacts weighed less than men who had not lived in the same house with a tuberculosis case, even when the comparison was limited to tuberculin-negative people in each group.[137]

5.12. Human Immunodeficiency Virus—HIV

Because of the importance of the cellular immune system in controlling infections with *M. tuberculosis*, it is not surprising that people infected with HIV are at increased risk of both reactivation and primary TB.[138] Linking registers for tuberculosis with those for AIDS has shown that there is a high degree of association, most marked among populations with overlapping risks for being infected with *M. tuberculosis* and HIV (e.g., intravenous drug users in inner cities or residents of some developing countries).[139–141] Populations with a high prevalence of infections with *M. tuberculosis* and HIV have extremely high rates of clinical tuberculosis.[12,138,142–145]

In 2003, Corbett et al. reported the results of an international survey designed to measure the burden of tuberculosis in the context of HIV. They found that approximately 9% of all new TB cases in adults 15–49 years of age were attributable to HIV infection, but the proportion was much greater in African and in some industrialized countries, notably the United States with proportions of 31 and 26%, respectively. An estimated 12% of all TB deaths in 2000 were attributable to HIV, while tuberculosis was the cause of 11% of all adult AIDS deaths. Co-infection with TB and HIV was estimated to be 11 million people, with the greatest proportions being seen in southern Africa. In South Africa alone there were 2 million co-infected adults.[12] Although southern Africa is currently suffering the brunt of these co-epidemics, other regions of the world also have growing numbers of co-infected people. Russia, India, and China and countries in Southeast Asia where the HIV epidemic is newer are home to huge and vulnerable populations.[146,147]

5.13. Size of Tuberculin Reaction

Several studies have shown that people who are most reactive to tuberculin are also most likely to develop tuberculous disease later on (Figure 5).[23,63,148,149] Up to 55 years ago, attention was focused on whether allergy to tuberculoprotein was beneficial or harmful; now, at least in epidemiological circles, principal interest lies in the ability of the degree of hypersensitivity to indicate whether or not the infecting organism was one of the tubercle bacilli, *M. tuberculosis* or *M. bovis*, or one of the usually benign nontuberculous mycobacteria.

The prognostic importance of degree of sensitivity to tuberculin was clearly shown in a study of more than 1 million white Navy recruits.[23] On entry into the Navy, recruits routinely received a skin test with PPD-S and also with a PPD prepared from a nontuberculous mycobacteria, either the so-called Battey or Gause strain, currently named *M. avium-intracellulare* or *M. scrofulaceum*, respectively. Cases of tuberculosis that developed among these men during their first 4 years of Navy service were identified. Recruits with less than 6 mm to 5 tuberculin units of PPD-S on entry had a low rate of tuberculosis subsequently; those with 6–13 mm reactions, an intermediate rate; and those with reactions of 12 mm or more, a high rate.

Both experimental and clinical evidence indicate that reactions to a homologous antigen are usually larger than cross-reactions to a heterologous antigen. Among recruits with intermediate reactions to PPD-S and larger reactions to either PPD-B or PPD-G, the subsequent case rate was essentially the same as recruits with less than 6 mm of induration to PPD-S, suggesting that their reactions to PPD-S were due to cross-reactions from nontuberculous mycobacteria.

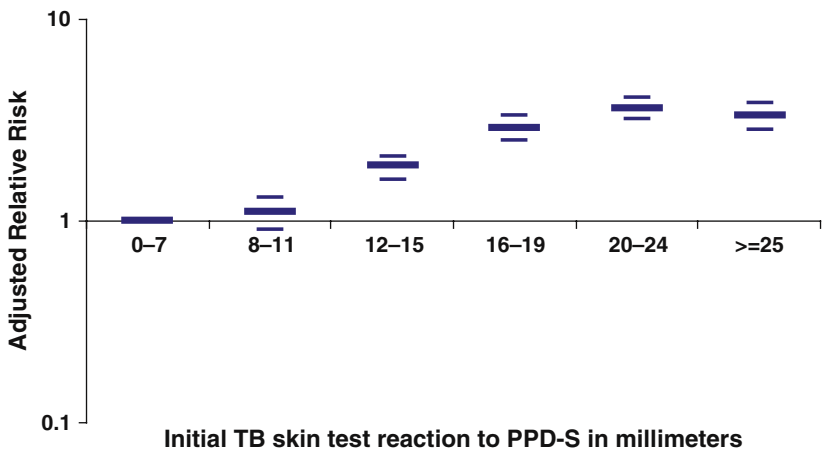


Figure 5. Adjusted relative risk of tuberculosis 0–15 years after the TB skin test by initial reaction size. Adapted from Radhakrishna et al.[149]

In contrast, recruits with intermediate reactions to PPD-S that were clearly larger than those to PPD-B or PPD-G had subsequent tuberculosis case rates that approached those of recruits with large reactions to PPD-S. These and similar findings support the current belief that the larger the reaction to a small or intermediate dose of tuberculin, the more likely it is that an individual has been infected with tubercle bacilli, and consequently the more likely that tuberculous disease will develop at some time in the future.[149] In HIV-infected populations, PPD reactivity continues to be associated with increased risk of tuberculosis disease.[150]

6. Mechanisms and Routes of Transmission

Tubercle bacilli are generally transmitted to humans in only two ways: by the inhalation of tubercle bacilli in droplet nuclei and by ingestion of contaminated milk. Tuberculin testing of dairy cattle and pasteurization of milk had nearly eliminated bovine tuberculosis in the United States and the possibility of its spread to man through dairy products, though recent outbreaks associated with contaminated Mexican cheeses are of concern.[151,152]

When an individual with tubercle bacilli in the sputum coughs, sneezes, talks, or sings, a spray of secretions may be expelled. The larger particles fall out of the air promptly and appear to be of little danger. Tiny particles dry promptly when exposed to air; the resultant droplet nuclei may float with the air currents for a long time and considerable distances.[153] When they are inhaled they can be trapped in the upper air passages or on the ciliated portions of the tracheobronchial tree, but may pass directly to the alveoli. Current evidence indicates that only droplet nuclei that carry tubercle bacilli and reach the alveoli are effective in infecting a susceptible host.

The importance of spread by direct contact, or by ingestion of organisms other than in infected dairy products, is probably slight. Theoretically, communicable droplet nuclei may be generated by manipulation of any material that contains significant numbers of tubercle bacilli. Cutaneous (soft tissue) tuberculosis can be the source of transmission, particularly if the wound is irrigated with a high-pressure system capable of creating aerosol and droplet nuclei.[154] Aerosol respiratory treatments, embalming, and autopsies have all proved their potential to introduce communicable droplet nuclei into the air.[155–158]

Direct inoculation is an unusual method to transmit *M. tuberculosis*.[159,160] Laboratory personnel are at risk from accidental skin punctures with contaminated equipment.[161] Fiberoptic bronchoscopes, inadequately sterilized between patients, have been implicated many times in directly inoculating patients with *M. tuberculosis*.[162,163] Rare instances of sexual transmission of *M. tuberculosis* or *M. bovis* have been suggested where an individual with genitourinary tuberculosis infects a partner.[164]

7. Pathogenesis and Immunity

After tubercle bacilli are inhaled or ingested, they are engulfed by macrophages and carried to the regional lymph nodes, occasionally passing into the bloodstream and thence to many organs of the body. The mycobacteria's waxy cell wall resists attack or digestion; initially it causes little or no reaction in the host tissues. Using mechanisms not fully understood, the engulfed mycobacteria function as intracellular parasites, subverting the phagolysosome so that it can continue to sustain itself with nutrients, but cannot be destroyed, at least temporarily.[165,166]

In a healthy host, the immune system eventually overcomes the mycobacterial interference and within 3–8 weeks, hypersensitivity to antigens develops.[167] Phagocytes that contain tubercle bacilli may then be encased by effector cells; the infected cells are then killed, resulting in foci with central caseous necrosis and a peripheral zone of epithelioid cells, lymphocytes, and fibroblasts. The range of possible outcomes is extremely varied. In many organs and tissues, blood-borne tubercle bacilli show no evidence of becoming established. Bone marrow, liver, and spleen are often involved, but infections in these organs are almost always controlled. More favorable sites for growth are the lungs, kidneys, meninges, and the epiphyseal portions of bone.[168] Even in favorable sites, however, the majority of lesions heal as scar tissue surrounds the caseous foci and tubercle bacilli. Some lesions become partly or wholly calcified. Although the organisms may remain viable for years in these scarred areas, it is likely that most of them eventually die.

In a small proportion of people, the balance tips against the host and in favor of the bacilli. Host resistance may be inadequate from the start, and the infection may progress to active disease; or after an indefinite period of quiescence and apparent healing, the organisms may again start to multiply and destroy tissue. The specific factors responsible for the inability to control the infecting organisms at either time are not yet known. It is known, however, that resistance is lowered in diseases (notably HIV infection) or therapy involving immunosuppression, diabetes, silicosis, and, as noted in Section 5.12, possibly by severe undernutrition.

Not all locations of disease are favorable for the transmission of infecting organisms, since organisms buried in most human tissues will never be excreted in the form of droplet nuclei small enough to be inhaled by another host.

Only in the lungs or especially the larynx is this likely to occur. Actively progressing caseous foci in the lung ultimately erode into a bronchus, the contents are expelled, and a cavity is formed. Tubercle bacilli flourish on the walls of a cavity and can readily pass with the expired air into the atmosphere, where the resulting droplet nuclei may survive to be inhaled into the lungs of another susceptible host.

As mentioned in Section 5, tuberculosis is unlike most other infectious diseases in the extreme variability between the time of infection and development of disease, ranging from a few weeks to a lifetime. When an individual develops tuberculosis disease it may represent endogenous reactivation or exogenous infection or reinfection. This issue is an important one and it is not surprising that it is also controversial.[169]

Individual strains of *M. tuberculosis* can be recognized by restriction fragment length polymorphism analysis (RFLP) or other techniques that identify unique genomic sequences.[170] Patterns made by the fragments, believed to be so unique that this procedure is referred to as "DNA fingerprinting," allow source and subsequent cases of TB to be identified that might otherwise would have been missed.[73,156,170–172] DNA fingerprinting has been useful in determining that the predominant causes of disease may differ in areas of low and high transmission of tuberculosis. Endogenous reactivation of dormant organisms after a period of latency in a previously infected person seems the more common mechanism in areas where the likelihood of infection is low, as in most of the developed world.[173–175] However, community-wide DNA fingerprinting studies suggest a much larger proportion of recent transmission, at least in urban settings, than previously suspected in developed countries.[171,176,177] Exogenous reinfection is disease caused by inhalation of tubercle bacilli by an individual already infected with TB when exposed to a second infectious case. There is evidence that in areas of ongoing TB transmission it is not uncommon for a person with successfully treated tuberculosis to become infected and ill with another strain, apparently inhaled sometime following treatment.[169,178]

There is evidence that initial tuberculosis infection does confer acquired resistance. A study carried out in Norway in 1927 noted that student nurses who began work with positive tuberculosis skin tests were significantly less likely to develop tuberculosis than those students with an initially negative reaction.[179] Similar findings, more recently, continue to support this observation.[180]

There is also evidence that the assumption of acquired resistance does not apply to everyone. One explanation could be that resistance is strain specific. Perhaps some people may not be able to mount a protective immune response against TB whether they have received immunization or been "naturally" exposed; these are people with inherently impaired immune systems.[169,181] What is known is that immunosuppression from HIV or chemotherapy leaves people vulnerable to reinfection despite successful and curative tuberculosis treatment.[180,182,183] It stands to reason that poor nutrition and chronic burdens of parasitic diseases could also leave many people potentially vulnerable in settings with high TB transmission. Greater understanding of the immunologic mechanisms required to acquire and sustain resistance in these settings will be necessary before effective vaccines can be developed.

8. Patterns of Host Response

The type of clinical disease that develops after infection depends to a considerable extent on the ability of host resistance to hold the multiplication of bacilli in check. Some lesions may be small and heal without ever producing symptoms or being recognizable by radiographic or pathological examination. Fortunately, many tuberculous lesions do tend to heal, even after reaching the symptomatic stage. Some, however, progress to a point where tissue destruction is extensive and results in progressive illness and, without treatment, death. The nature of the disease that results from reactivation of an initial lesion or lesions depends most of all on their location. The manifestations of tuberculosis of various organs differ so markedly that it was not until Koch demonstrated the causative organisms that they were accepted as having a common etiology.

While pulmonary disease is the most common presentation, extrapulmonary disease is not unusual. Those who progress rapidly to disease after initial infection have a greater risk of extrapulmonary manifestations, particularly pleural disease. There is evidence that extrapulmonary tuberculosis is more likely to occur in people with immunosuppression, either innate or acquired.[83,184,185] Children appear more likely to develop extrapulmonary tuberculosis, apparently because of immature immunity.[83] A recent study using routine national surveillance data, designed to identify risk groups in the United States, was able to determine that women, non-Hispanic blacks, and HIV-positive TB cases were more likely to manifest extrapulmonary disease.[186] The proportion of tuberculosis cases with extrapulmonary disease increased from 16% in 1990 to 20% in 2000. While the overall rate of new TB cases continues to decline in the United States since national reporting began in 1953, the annual decrease in TB cases has slowed from an average of 7.1% (1993–2000) to the current average of 3.8% (2001–2005), according to CDC. This may be partially explained by the increasing proportion of foreign-born indi-

viduals with TB, who also have disproportionate rates of HIV infection.[8]

9. Control and Prevention

9.1. General Concepts

The behavior of TB in populations suggests that evolution of host resistance may be important in reducing the incidence and deadliness of the disease. When TB enters a naïve population, case rates and mortality are initially very high, then gradually decline over a number of generations. Historians have noted that the decline in tuberculosis in many countries appears to have started before the discovery of the tubercle bacillus, and hence prior to any measures specifically designed to control the disease. Others have pointed out, however, that improved living and working conditions brought about by the sanitation and social reform movement of the 1800s might well have helped produce a decline in tuberculosis.[4] In particular, improved ventilation and daylight in houses and places of employment should have decreased the probability of becoming infected, while shorter work weeks and improved nutrition should have increased resistance to disease. It seems likely that improvements along these lines contributed to the decline of tuberculosis.

Tuberculosis control measures among humans fall into four major categories: decontamination of air and milk, case-finding and chemotherapy, treatment of latent TB infection, and vaccination. Each has its advantages and disadvantages, and each has a place under the appropriate circumstances. A simple model serves well to illustrate how each of these control measures affects the tuberculosis problem.[187] The major elements of the model are shown in Figure 6, adapted from Shirley Ferebee's model first published in 1967. Missing from the figure's portrayal of a 1-year cycle is the reduction of the uninfected and infected populations by deaths and the addition to the uninfected population by births.

In any given year, a certain number of cases develop from previously infected people. Each of these cases infects others, some of whom develop tuberculosis during that year and others of whom are added to the infected group for the subsequent year. These new cases in turn infect others, who are also added to the infected group. As you will see in the following discussion, *if* the effectiveness of tuberculosis control could be maintained, tuberculosis would eventually disappear, although this would require many years.

9.2. Decontamination of Air and Milk

When pulmonary tuberculosis patients are identified, precautions should be aimed at preventing air from becoming a vehicle of infection. The formation of infectious particles can be markedly reduced merely by covering the mouth and nose when coughing or sneezing. The patient should be taught to cover the mouth and nose with disposable tissues when coughing or sneezing in order to trap droplets before they can evaporate and become free-floating droplet nuclei. Initiating treatment rapidly reduces the infectivity of patients and is the most important intervention for protecting others from infection when TB patients are hospitalized. There should be good ventilation in the room without recirculation of air; preferably, the air should be exhausted directly to the outside and the room kept under negative pressure. Ultraviolet irradiation of the upper air or of recirculated air is also helpful. High-efficiency particulate (HEPA) masks or respirators are strongly encouraged for use by all personnel caring for infectious tuberculosis patients.[188,189]

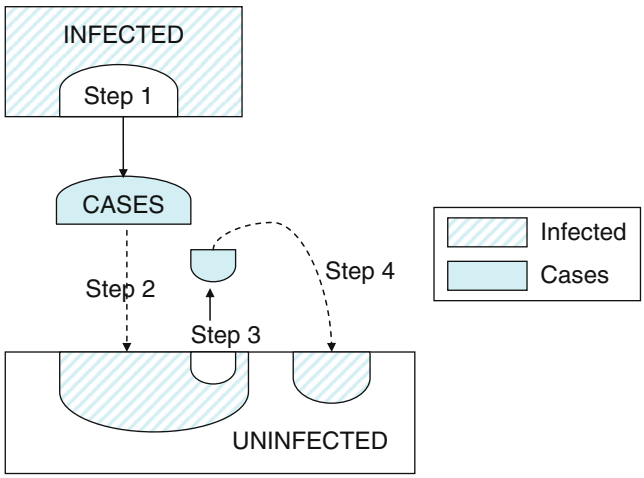


Figure 6. Schematic illustration of annual cycle of development of tuberculosis infection and disease. Adapted from Ferebee.[187]

In the occasional instances when explanation and persuasion fail and a patient with infectious tuberculosis refuses to take the necessary steps to protect others, it is possible in the United States and some other developed countries to use legal means to enforce isolation.[190,191] These measures are not accepted universally.[192] In areas of endemic tuberculosis there is often no cultural imperative to quarantine contagious individuals, as it is assumed that many people are already infected and thus not at risk or are protected by the BCG vaccination administered at birth so that forced isolation is believed to be both unnecessary and cruel.

The major means of keeping milk from being a vehicle of infection are periodic tuberculin testing of dairy cattle, slaughter of reactors, and pasteurization of milk. Although tuberculosis among cattle has been nearly

eliminated from the United States and milk-borne tuberculosis has become rare, this near success has led to diminished funding for the elimination program, laxity in tuberculin testing, and renewed interest in drinking raw milk, all of which could lead to milk again becoming a source of tuberculous infection. Recent cases of *M. bovis* in children that consumed non-pasteurized Mexican cheeses in New York City underscore the importance of vigilance in this area.[151]

If by some miracle both air and milk could be kept completely free of tubercle bacilli, the cycle of tuberculosis shown in Figure 6 would be blocked at Step 2. Tuberculosis would continue to develop among the previously infected population until all infected people had died.

9.3. Case-Finding and Chemotherapy

Similarly, in an ideal but unfortunately imaginary world, case-finding endeavors would identify every case before it became infectious and chemotherapy would be completely effective in preventing future infectiousness. Such perfection would also block the flow of tuberculosis at Step 2 in the model shown in Figure 6. As noted before, cases would continue to develop from the pool of infected people until all had died of TB or other causes.

Case-finding in developed countries is now difficult because the prevalence of cases has become so low that mass screening procedures are unproductive and the index of suspicion of most medical practitioners is low. In developing countries, where mass screening methods could be more useful but usually cannot be afforded, microscopic examination of stained specimens of sputum from people with chronic cough has been shown to be effective in identifying the most infectious cases; the impact on disease incidence has been mixed. In Peru, this strategy has led to a sharp decline in incidence, while in Vietnam it has not.[193] In both developed and developing countries, the most appropriate combination of diagnostic modalities—medical history, skin testing, chest radiography, and bacteriology—will need to be selected after reviewing the local tuberculosis problem and the resources to combat it.[194,195] In settings with a high prevalence of HIV infection, there is evidence that active TB case-finding, with initial screening tools as basic as a questionnaire, can yield high proportions of active cases.[141,196–199] Chemotherapy has become so effective that virtually every patient with organisms sensitive to the standard antituberculosis drugs could be cured if the necessary medications were taken conscientiously. Not only can the disease be cured, but patients are also quickly rendered noninfectious by the generally accepted chemotherapeutic regimen. This consists of the daily administration of four drugs—isoniazid, rifampicin, pyrazinamide, and ethambutol—for 2 months, and two drugs—isoniazid and rifampicin—for another 4 months.[200]

While this regimen may be simple from a medical point of view, the toxicity, duration of the regimen, and competing interests and needs can cause some patients to discontinue treatment prematurely or take it irregularly.[201–206] Interruption and discontinuation of treatment can lead to the resumption of TB transmission, and in some cases to the development of drug resistance.[207–210] The emergence of drug-resistant organisms necessitates expert administration of expensive and toxic drugs. Even then, the prognosis is often unfavorable. Treatment success for patients with multidrug-resistant tuberculosis requires 18–24 months of therapy and treatment success ranges for 45–75%.[211,212]

In 2000, in the United States, 56% of the 16,372 reported cases had pulmonary tuberculosis with known positive sputum; it is theoretically possible that with further examinations, all of the pulmonary cases (80%) might be classified as communicable. Of the 15,784 with any form of tuberculosis who were started on chemotherapy in 2000, 1,103 were known to have died (from any cause). Fewer than 85% of the others were known to have completed a recommended course of therapy.[8] Although it has improved over the years, even better performance is needed if the United States hopes to eliminate tuberculosis by case-finding and chemotherapy within the next few decades.

Directly observed therapy (DOT) is a strategy to promote treatment adherence and to reduce the development of drug resistance.[213,214] There have been many adaptations and enhancements to the strategy, but the basic idea is to provide support and supervision to TB patients during the long course of their treatment.[215–222] Studies carried out in Singapore and Hong Kong demonstrated that intermittent therapy—twice or three times weekly—was as effective as daily medication, making DOT more practical and affordable.[223,224] There is controversy about the necessity of literally observing patients take medication, but there is consensus in the TB control community that the other four points in the system defined by WHO as directly observed therapy short-course (DOTS) (Figure 7) are necessary and appropriate for TB control.[225] Evidence of the effectiveness of DOTS is that although global TB incidence rate is slowly rising, prevalence and death rates are falling.[56] Attention to administrative details and directly observed intermittent regimens can all contribute to better patient acceptance of treatment, to a high rate of cure, and to prevention of the emergence of drug-resistant strains.[217,220,226]

Five Components of the DOTS Strategy

- Government commitment to sustained TB control activities.
- Case detection by sputum smear microscopy among symptomatic patients self-reporting to health services.
- Standardized treatment regimen of 6-8 months for at least all confirmed sputum smear positive cases, with directly observed treatment (DOT) for at least the initial 2 months.
- A regular, uninterrupted supply of all essential anti-tuberculosis drugs.
- A standardized recording and reporting system that allows assessment of treatment results for each patient and of the TB control program overall.

Figure 7 World Health Organization strategy of directly observed therapy short-course.[221,222]

9.4. Preventive Therapy

Following the introduction of isoniazid as a chemotherapeutic agent against tuberculosis, Edith Lincoln, then the dean of the tuberculosis experts in the United States, noted that children with primary tuberculosis treated with this drug showed a much lower incidence of serious tuberculous complications than had been noted with other medications and suggested that a controlled trial of the effectiveness of isoniazid in preventing these complications be undertaken.[79] The ensuing trial among 2,750 children with asymptomatic primary tuberculosis or recent tuberculin conversions showed treatment with isoniazid to be remarkably effective, producing a 94% reduction in tuberculous complications during the year of preventive treatment and a 70% reduction over the subsequent 9-year period. Fears that the preventive use of isoniazid would lead to the emergence of isoniazid-resistant strains of tubercle bacilli have not been borne out.[79]

At least 21 controlled trials of preventive therapy with isoniazid involving more than 136,000 subjects have now been reported.[227,228] Among 14 trials with more than 1,000 subjects, the median reduction in tuberculosis from preventive treatment was 60% during the periods of observation, which ranged from 1 to 13 years. The range of effectiveness in these 14 trials was 25–88%. The trials with low effectiveness included one that used small doses of isoniazid and one in which adherence was very poor. It should be noted, however, that these estimates are based on the total study populations regardless of how well medication was taken. People with high adherence tended to obtain greater protection than the preceding median values indicate. Among people who adhere well to the treatment regimen, 12 months of medication has been shown to be more effective than 6 months.[229] Studies among tuberculosis contacts and Alaskan villagers show that 9 months of treatment is optimal.[79,230]

Some degree of protection can be expected to be long-lasting. Any diminution caused by isoniazid in the numbers of tubercle bacilli harbored by the host increases the probability that host resistance can control any organisms that may remain. In addition, it has been shown that a treated infection in guinea pigs confers some resistance against subsequent reinfection.[231] Protection in humans has been shown to persist for at least 10–19 years.[79,232]

The principal appeal of treatment for latent TB infection lies in its ability to interrupt the chain of infection at Step 1 in the model shown in Figure 6. Universal application to reactors and perfect effectiveness of this preventive procedure could theoretically eradicate tuberculosis almost at once. While realism does not allow hope of even approaching such an outcome, whatever effectiveness preventive treatment may have affects every step in the model and not merely the later ones. Of considerable current interest is the evidence from observational and controlled studies that preventive treatment with isoniazid not only reduces the risk of tuberculosis among HIV-infected people but may also delay the onset of symptoms of AIDS.[138,228,233] Treatment with isoniazid following a full course of curative TB therapy provided continued protection in HIV-positive people.[234]

Other preventive regimens have been evaluated, largely to determine whether shorter regimens using other antimycobacterial drugs are equivalent to therapy with isoniazid. A 2-month drug regimen of pyrazinamide and rifampin had promising results in trials enrolling HIV-infected adults, but for reasons still not fully understood, the increased hepatotoxicity of the regimen was responsible for the deaths of HIV-uninfected individuals in the United States, curtailing its use.[235–238] The use of rifampin is recommended when it is known or suspected that the infection is due to an isoniazid-resistant strain of TB, but isoniazid continues to be the backbone of the treatment for latent tuberculosis infection.[36]

A comprehensive review of 11 randomized controlled trials of TB preventive therapy regimens, evaluating a total of 8,130 HIV-infected participants, determined that TB preventive therapy, regardless of regimen, was associated with a significant reduction in the subsequent development of active tuberculosis. The effect was more pronounced in those people with a positive TB skin test than those with a negative test. The length of the protective effect was variable. Each of the drug regimens was equally efficacious, although those individuals who received isoniazid were less likely to suffer adverse effects.[239] It is recommended that a person who tests positive for HIV be tuberculin tested; if there is

induration of 5 mm or more to 5 tuberculin units of tuberculin PPD, preventive therapy with isoniazid is strongly advised.

9.5. Immunization

Immunization against tuberculosis is currently limited to the use of BCG (bacille Calmette–Guérin), an attenuated organism named for the two French investigators responsible for its development.[4] Working with *M. bovis* in 1908, they added bile to their culture media in an effort to obtain well-dispersed suspensions of the organisms. As an unexpected result, they noted that the organisms cultured in this way lost virulence for laboratory animals. After 13 years of subculturing and testing, they became convinced that they had developed a strain of organisms that would remain avirulent and could be used to immunize humans against tuberculosis.

BCG has probably had more, larger, and possibly better-controlled trials of its usefulness and effectiveness than almost any other preventive measure. Despite this, there is marked disagreement about its effectiveness. A meta-analysis of 15 of the 16 controlled trials of intracutaneous BCG vaccination concluded that the average efficacy was 50% (range –56 to +80%).[240] It was concluded that much of the variability was due to latitude, a surrogate for the prevalence of nontuberculous mycobacterial infections, with the least protection at lower latitudes. Although the possibility was discounted that some of the variability in results might be associated with differences in BCG strains, characteristics of BCG vaccines can change with continued cultivation. To some, this seems a likely explanation for the marked differences observed among the results of various trials done around 1950 with different substrains of vaccines that were then more than 25 years removed from the original parent strain. With the lapse of 40 or more years since these trials, reliable information is lacking on the effectiveness of currently available strains. That some strains of BCG do differ in efficacy is indicated by the findings in each of two case–control studies of marked differences in effectiveness when one vaccine was substituted for another[241] and in a recent controlled trial of two different BCG strains.[242,243] It has recently been shown that currently available BCG strains differ in their genetic makeup.[244] Unfortunately, there is currently no way other than long-term studies to assess efficacy of the strains. Postvaccinal tuberculin sensitivity is not related to efficacy and animal models are variable and cannot be used to predict responses in humans.[241]

Because of uncertainties regarding the efficacy of most BCG vaccine strains, the decision on the use of vaccination must depend in part on the availability of other means of tuberculosis control. In much of the world, the decision is not difficult. In countries with a very low infection rate, such as the United States, with a readily available alternative program of tuberculosis control by case-finding, chemotherapy, and preventive therapy, there is very little need for the kind of protection that vaccination can give and considerable reason to protect the usefulness of the tuberculin test.[245] As a result, in the United States BCG vaccination is recommended only for tuberculin-negative people at a high risk of becoming infected for whom no other method of tuberculosis control is feasible. Its use is contraindicated in people with or at high risk of being infected with HIV, because the vaccine can cause localized or disseminated disease in immunocompromised people.

In many other countries, there is clearly a high risk of tuberculosis infection, especially in infants, and little prospect of effective control except by BCG vaccination. In such situations, vaccination programs must be continued, except among people with known HIV infections. Preliminary tuberculin testing is not needed for vaccination of newborn infants. Nor is skin testing necessary for vaccination programs aimed at older people, since it has been shown that vaccination of tuberculin-positive people and people with tuberculosis did not result in demonstrable harm.[246]

The vaccine is inexpensive, its administration is simple, and vaccination probably does not have to be repeated. Serious complications are very uncommon.[247,248] However, vaccination does produce localized ulceration that can persist for several months and may be accompanied by local lymphadenopathy. A potentially important side effect is the production of sensitivity to tuberculin. In areas with a high risk of infection, most people will become tuberculin reactors in the course of time, and the premature production of tuberculin sensitivity by vaccination is of little consequence. In areas with a low risk of infection, the help that the tuberculin test can give diagnostically and prognostically constitutes an added contraindication of BCG vaccination. Also in these areas, the reports of a long-term risk of lymphomas associated with vaccination in two of three trials should not be ignored.[243,249]

The role that effective immunization might play in the control of tuberculosis is also indicated by the model in Figure 6. As was the case for case-finding and chemotherapy (Section 9.3), the effect of immunization is on Step 2, namely protecting the uninfected population against development of disease when exposed to infectious cases. Universal application of a vaccine with 100% efficacy would not eradicate tuberculosis until all infected reactors had died.

Do you want to indicate the length of protection by use of BCG as it provides protection against spread in children but not infections?

10. Unresolved Problems

Tuberculosis is increasing today at a worldwide average rate of 1% a year. There are many important issues that need to be resolved to turn the tide and successfully control tuberculosis. Perhaps the most important, the element that is essential but not sufficient, is political will. The eradication of smallpox is a proud example of what can be accomplished in seemingly impossible circumstances when there is political will, much as the English proverb says, "Where there's a will, there's a way."

Reducing the risk of infection and subsequently the risk of developing TB disease are problems for which there are solutions, if there is the will to carry them out. Improving the general living conditions of millions of impoverished human beings who populate this earth would reduce the risks of infection by diminishing exposure from overcrowding and bolstering immune response through improved nutrition. Instituting the universal habit of covering the mouth with a cloth or tissues, when coughing, or coughing into the elbow will reduce transmission. Instituting and enforcing the isolation of suspected TB patients and simultaneously taking measures to protect health care staff will further reduce transmission. The risks of transmission come from those TB cases that are not yet recognized and that have not started treatment. There is ample evidence that once treatment begins, the risks of TB transmission are small to nil; the risks of transmission come from individuals that are not yet suspected of having TB.[48,250]

Reducing the risk of infection with HIV will help to reduce the pool of individuals with exceptional vulnerability to tuberculosis infection and disease. Once infected, all individuals with HIV should have the opportunity to learn their infection status, and must have access to medications that will prevent opportunistic infections (including tuberculosis), treat their HIV infection, and reduce their risk of dying.

Development of a TB vaccine with known efficacy would be of great use. Protecting individuals at birth from the risk of infection with tuberculosis would be ideal. Currently, there is uncertainty about the efficacy of BCG strains in use. BCG has been protective in some populations, so it will be important to fully understand which BCG strains are protective and why.

Development of a sensitive test that could easily and reliably identify infected people who still harbor living tubercle bacilli would permit truly targeted prevention of active tuberculosis; only those with active infection would require, and benefit from, treatment. The present tuberculin skin test and serological tests fail on two counts: (1) positive reactions can be caused by mycobacteria other than *M. tuberculosis* or *M. bovis* and (2) reactions from a tuberculous infection may persist long after the infecting organisms have succumbed to the host's defenses. Improvement of the tests on either count would make both diagnosis and preventive therapy much more efficient. Long-term follow-up studies of people who have positive interferon-gamma release assays are needed to determine the prognostic potential of these new immune-diagnostic tests.

The length of an effective course of TB treatment, despite the name "short-course," is still too long. Six months of treatment is expensive and difficult to maintain for TB patients and their care providers. Although cure rates of 95% are encouraging, the risk of failure which seems small when expressed as a percentage still leaves adequately treated patients as a high-risk group. This becomes obvious when a failure rate of 5% is expressed as 5,000/100,000. The development of truly short, effective, and non-toxic regimens will be essential to improve the rates of treatment completion and cure. The availability of such drugs is only half of the story; TB patients must have access to them as well. Clinics must be within reach and medications must be free of charge.

Drug-resistant tuberculosis is a major control issue in some parts of the world. Ironically, how to prevent tubercle bacilli from becoming resistant has been known for decades. Completing an effective, multidrug regimen is the best way to keep the emergence of drug-resistant organisms to a minimum. Too often, drug resistance is the result of a lack of TB control due to a lack of finances and poor administration. Without consistent support, patients may be selective in which medications they take, leading to the development of strains no longer killed by the drugs used in standard short-course therapy. The same result occurs when a clinician prescribes inappropriate or incomplete drug regimens and when drug supplies are interrupted, because patients' funds run out or from inadequate distribution systems.[210,251–253] The resultant irregular or ineffective treatment allows drug-resistant organisms to emerge, to spread to others, and to be treatable only with more toxic and longer regimens. Even in the best of circumstances, treatment outcomes are frequently poor for those afflicted with multidrug resistance—a strain of TB that is not killed by either isoniazid or rifamycin.[254]

The tuberculosis pandemic has received international attention, and along with HIV and malaria has become the catalyst for unprecedented collaboration between the private and public sectors.[255,256] Tuberculosis control requires not only financial but human resources. It has finally been recognized that the only realistic method of garnering these resources is through creative collaboration between the public sector, nongovernment organizations, and corporations. The World Health Organization followed such a model when

it created the Global Partnership to Stop TB in 2000. Comprised of international organizations, countries, donors from the public and private sectors, governmental and nongovernmental organizations, and individuals, Stop TB now boasts 400 Partners from around the world. One of the Partnership's great accomplishments has been the development of the Global Drug Facility that helps countries obtain, manage, and distribute affordable and good-quality drugs. Most recently the Stop TB Partnership has published the Global Plan to Control TB, an ambitious and meticulously detailed blueprint for actions needed to reduce TB prevalence and death by 50% by 2015.[257] Founded in 2001, the Global Fund to Fight AIDS, Tuberculosis and Malaria is a unique, private financing mechanism that attracts money from governments, foundations, corporations, and individuals that it then manages and disperses through existing agencies and by awarding grants. Only time will tell whether our will is strong enough to see our way to truly effective international TB control.

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CHAPTER 41

Nontuberculous Mycobacterial Infections

Charles L. Daley

1. Introduction

The nontuberculous mycobacteria (NTM) comprise over 125 different species that are widely distributed throughout the environment.[1] Over the decades the NTM have been referred to as mycobacteria other than tuberculosis (MOTT), atypical mycobacteria, and environmental mycobacteria. Despite their frequent isolation in the environment and human specimens, the NTM were not widely recognized as a cause of human disease until the late 1950s. Since that time, the number of new species of NTM has grown dramatically primarily related to the availability of DNA sequencing, which has allowed identification of new species simply by detecting relatively small differences in DNA sequences. In addition to the proliferation of new species, the rate of disease related to NTM has also increased in some areas.

NTM have several features that distinguish them from organisms in the *Mycobacterium tuberculosis* complex. The NTM display a wide range of pathogenicity and unlike *M. tuberculosis*, they are not transmissible from human to human. Many of the pathogenic NTM are relatively drug resistant compared with *M. tuberculosis*, and thus, they are difficult to treat with high rates of treatment failure and recurrence. Because of our poor understanding of the transmission and pathogenesis of most NTM infections, we have little insight into how to prevent these infections and thus our public health strategy to control disease caused by these organisms is limited.

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2. Historical Background

NTM have been recognized since the late nineteenth century when "tuberculosis" in birds was described prior to the isolation of the human tubercle bacillus.[2] As these organisms did not cause characteristic disease when inoculated into guinea pigs, they were recognized as distinct from *M. tuberculosis* and were not believed to cause disease in humans. The recognition that these organisms caused human disease was described as early as 1893,[3] and numerous reports supporting their role as a true pathogen appeared subsequently.[4,5]

By the 1950s, pulmonary disease due to NTM became more commonly recognized and accounted for approximately 1–2% of admissions to tuberculosis sanatoriums in the southeastern United States.[6] In 1954 Timpe and Runyon classified these "atypical" mycobacteria into four groups based on simple culture characteristics: (1) photochromagens (group I), which develop yellow or orange pigment when exposed to light; (2) scotochromagens (group II), which form orange-yellow pigment in the dark; (3) nonchromogens (group III), which are unpigmented; and (4) rapid growers (group IV), which grow on culture media within 7 days. This latter group is distinguished from the slow-growing mycobacteria, which usually takes 3–6 weeks to grow.

Interest in NTM increased when HIV-infected patients began to develop disseminated infections due to various NTM species, particularly *M. avium*.[7–11] As antiretroviral drugs became available, the incidence of disseminated MAC decreased but we are now seeing an increase in the number of cases of pulmonary disease due to NTM particularly among older Caucasian women.

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3. Methodology

3.1. Sources of Data

Infections due to NTM are not reportable to public health authorities in most countries, including the United States. Therefore, surveillance data are seldom available. Most studies that have described the epidemiology of NTM can be broadly divided into three types: (1) skin test studies; (2) laboratory-based microbiologic studies; and (3) studies with clinical correlation.[2] The latter studies are the most useful because they combine information from the mycobacterial laboratory and the clinician's assessment; however, there are relatively few of these studies available.

3.2. Laboratory Diagnosis

3.2.1. Isolation and Identification of NTM. The bacteriological diagnosis of NTM is based on isolation of these organisms from diagnostic specimens using standard mycobacteriology laboratory techniques. All cultures should include both solid and broth media for detection of mycobacteria and a semi-quantitative reporting of colony counts is recommended.[1] Several mycobacterial species, including *M. haemophilum, M. genavense, M. avium subsp. paratuberculosis,* and *M. ulcerans*, require special supplementation for recovery on culture.

The optimal temperature for most cultures of NTM is between 28 and 37°C although some species require either higher or lower temperatures for optimal growth.[1] Most NTM grow within 2–3 weeks on subculture although *M. ulcerans* and *M. genavense* may require at least 8–12 weeks and rapidly growing mycobacteria usually grow within 7 days of subculture.

Species-specific identification of NTM has become increasingly important because of the variation in antimicrobial susceptibility and treatment options. Identification can be based on phenotypic, chemotaxonomic, and molecular methods.[1] NTM can be categorized by their growth rate and pigmentation although these characteristics are not specific enough for final speciation. Isolates which form colonies within 7 days of subculture are referred to as rapidly growing mycobacteria whereas those that take longer than 7 days to form colonies are referred to as slowly growing mycobacteria. As noted earlier, the NTM can be divided into three groups based on the production of pigment. In addition, biochemical testing can be used to help speciate the NTM. However, none of these procedures are sufficient to differentiate all NTM, particularly some of the newly identified species.[12–14]

Along with traditional techniques, the modern clinical laboratory includes a broad variety of rapid methods for mycobacterial speciation including nucleic acid probes, polymerase chain reaction and other amplification methods, high-performance liquid chromatography (HPLC), and nucleic acid sequencing.[15–24]

The commercially available AccuProbe technology (Gen-Probe, San Diego, California) is currently recommended for identification of *M. tuberculosis* complex, *M. avium* complex (as well as *M. avium* and *M. intracellulare* separately), *M. kansasii,* and *M. gordonae*. This method, which targets 16S rRNA in the organism, requires a substantial amount of bacteria, and thus, it takes at least 3 weeks for cultivation on agar medium or 10–12 days in a liquid medium to achieve sufficient growth for this test.

HPLC, which analyzes the chromatographic profile of the mycolic acids extracted from the bacterial cell wall, is a highly sensitive method that can detect at least 63 chromatographic patterns representing 73 mycobacterial species.[19,20,22,25] A much broader range of species can be reliably identified by combining information from the HPLC test results with other data, such as growth requirements, growth rate, colonial morphology, and pigmentation.

Identification of mycobacteria by 16S ribosomal DNA sequencing[17,21,23,24] provides more accurate determination of the species although not all species can be differentiated by this method. The procedure includes disruption of the bacterial cells, followed by amplification of a 1 kb fragment of 16S ribosomal DNA. The species identification is based on a comparison between the sequences obtained and those in the libraries of already known species. The supplies for the test and the libraries are commercially available in an automated format (MicroSec 500 16S rDNA Bacterial Sequencing Kit system from Applied Biosystems, Foster City, California).

3.2.2. Drug Susceptibility Testing. There is continued controversy regarding the clinical usefulness of drug susceptibility testing in the management of patients with NTM. This controversy stems from the fact that the in vitro results do not correlate well with clinical outcomes for some mycobacterial species.

For slowly growing mycobacteria there is no single susceptibility method that is recommended for all species. For MAC, a broth-based culture method with both microdilution and macrodilution methods is considered acceptable.[1] Initial isolates should be tested for clarithromycin as well as isolates from treatment failures and relapses, those who have taken macrolides previously, and AIDS patients who develop bacteremia on macrolide prophylaxis.[1] Testing of other drugs may be useful when trying to select additional drugs for treatment of patients with MAC, especially in patients

who are failing therapy, have relapsed, or have macrolide-resistant disease.

Isolates of *M. kansasii* should be tested to rifampin as resistance to rifampin is associated with treatment failure/relapse. If rifampin resistance is documented additional drugs should be tested including amikacin, fluoroquinolones, macrolides, ethambutol, sulfonamides, and isoniazid.

Broth microdilution MIC determination for susceptibility testing is recommended for rapidly growing mycobacteria and inclusion of the following drugs is recommended: amikacin, kanamycin, tobramycin, imipenem, doxycycline, clarithromycin, azithromycin, trimethoprim/sulfamethoxazole, augmentin, cefoxitin, ciprofloxacin, moxifloxacin, tigecycline, and linezolid.

3.2.3. Serological and Immunologic Tests. Delayed-type hypersensitivity reactions to mycobacterial antigens can be detected in patients who have been infected with NTM. Similarly, most patients with evidence of NTM disease also demonstrate serological responses to infection.[26] A recent study evaluated an enzyme immunoassay (EIA) kit for the diagnosis of pulmonary MAC disease.[27] The assay detects serum IgA antibody to glycopeptidolipid core antigen specific for MAC. Antibody levels were measured in 70 patients with MAC lung disease, 18 with MAC "contamination", 37 with pulmonary tuberculosis, 45 with other forms of lung disease, and 76 healthy controls. Significantly higher serum IgA antibody levels were detected in patients with MAC pulmonary disease compared to other groups ($p < 0.0001$). Significantly higher IgA concentrations were also detected in patients with nodular bronchiectasis disease compared to fibrocavitary disease ($p < 0.05$), and there was a correlation between antibody levels and the radiographic extent of disease ($p < 0.05$). The sensitivity of the assay was 84.3% and the specificity 100% for detection of MAC lung disease.

A first-generation interferon-gamma release assay (QuantiFERON TB Test, Cellestis) included MAC PPD in the test, but data regarding the utility of this assay in the diagnosis of MAC lung disease are lacking. In one of the first studies evaluating the QuantiFERON test, reactivity to MAC PPD was associated with discordance between the tuberculin skin test and the QFT-TB results, suggesting underlying MAC infection was causing false-positive tuberculin skin test results.[28] In a study that performed QFT and skin testing with PPD-B in injection drug users, the authors reported poor concordance between the tests (63%)[29]. Additional studies will be needed to access the clinical utility of these types of assays.

4. Biological Characteristics of the Nontuberculous Mycobacteria

Mycobacteria belong to the order of Actinomycetales.[30–32] In 1998, there were 71 described species of the *Mycobacterium* genus but today there are at least 125

Table 1. Slowly and Rapidly Growing NTM Reported to Cause Disease in Humans

Slowly growing mycobacteria		Rapidly growing mycobacteria	
M. alsiense	*M. lentiflavum*	*M. abscessus*	*M. confluentis*
*M. avium**	*M. malmoense*	*M. chelonae*	*M. cosmeticum*
M. asiaticum	*M. marinum*	*M. fortuitum*	*M. elephantis*
M. bohemicum	*M. nebraskense*	*M. mucogenicum*	*M. goodii*
M. branderi	*M. parmense*	*M. peregrinum*	*M. hassiacum*
M. chimaera	*M. parascrofulaceum*	*M. porcinum*	*M. immunogenum*
M. celatum	*M. palustre*	*M. senegalense*	*M. mageritense*
M. conspicuum	*M. saskatchewanse*	*M. bolletii*	*M. novocastrense*
M. doricum	*M. scrofulaceum*	*M. brumae*	*M. septicum*
M. florentinum	*M. sherrisii*	*M. canariasense*	*M. thermoresistible*
M. genavense	*M. shimodei*	*M. conceptionense*	*M. wolinskyi*
M. haemophilum	*M. simiae*		
M. heckeshornense	*M. szulgai*		
M. heidelbergense	*M. tusciae*		
M. intermedium	*M. triplex*		
M. interjectum	*M. ulcerans*		
M. intracellulare	*M. xenopi*		
M. kansasii			
M. kubicae			

*Subspecies: *avium, colombiense, silvaticum, hominissuis, paratuberculosis.*
References: 12,30,32,33,46,86,114.

separate species that have been reported.[30,32–34] The rapid expansion in the number of mycobacterial species is in part related to the availability of new methods that can differentiate new species at the molecular level.

The pathogenicity of the various NTM species varies greatly, from organisms that are seldom pathogenic to humans such as *M. gordonae,* to those that are almost always disease producing, such as *M. kansasii*. The biologic reason for this wide spectrum of pathogenicity is poorly understood.

As noted previously, NTM are typically divided into rapidly and slowly growing mycobacteria based on their ability to produce visible colonies on solid culture medium at the optimal conditions in less or more than 7 days (Table 1). In addition, the various species can be classified by the presence or absence of pigmentation as described in the Runyon and Timpe classification. Based on isolation from patients in whom the isolate was identified as a pathogen, at least 30 organisms among slowly growing mycobacteria are considered potential pathogens. In addition, other slow-growing mycobacteria that are not considered definitely "pathogenic" for humans can be found in diagnostic specimens often from environmental contamination. Similarly for rapid growers, a minority of the known species have been reported to cause disease in humans.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Cutaneous delayed-type hypersensitivity to mycobacterial antigens has been used to assess the frequency of infection with *M. avium* and *M. intracellulare* and has demonstrated that a substantial proportion of adults have had prior exposure to MAC. Between 1943 and 1959, 22,000 nursing students were tested with 5 tuberculin units of PPD-S.[35] Students who did not react to 5 tuberculin units were retested with 250 units to detect reactivity to cross-reacting antigens. Sixty-five percent of students who came from the southeastern United States reacted to the higher dose of tuberculin compared with only 29% of those from other areas of the country, suggesting a high rate of infection to MAC and possibly other NTM species. Similarly, skin test surveys of U.S. Navy recruits in the 1960s documented higher rates of reactivity in those from the southeastern United States (56%) than from the northern states (15–25%).[36]

Microbiological survey data from state laboratories in the early 1980s estimated a prevalence of NTM infection of 1–2 cases per 100,000 population.[37] A similar survey from 1993 to 1996 reported an annual case rate of 7–8 per 100,000 documenting an increase in isolation of NTM when compared to the previous survey.[38] Because these laboratory-based surveys did not include clinical information, it is likely these types of studies overestimate the prevalence of disease.

The most useful studies combine clinical and microbiologic data to provide estimates of disease rates. The incidence of pulmonary NTM was studied prospectively in France between 2001 and 2003 at 32 sentinel surveillance sites: 262 patients met the ATS criteria for disease (see below).[39] *M. avium* complex was the cause of disease in almost half of the patients followed by *M. xenopi* in 25%, *M. kansasii* in 13%, and rapidly growing mycobacteria in 10%. The overall incidence was estimated to be stable and approximately 0.72–0.74 per 100,000; however, the method for calculating the incidence rate likely underestimated the true incidence.

A recent population-based study from Ontario Province in Canada documented a prevalence of NTM infection of 14.1 per 100,000 in 2003 ($p < 0.0001$).[40] The most common isolate was MAC with a prevalence of 8.4 per 100,000. Thirty-seven percent of 200 patients evaluated at one hospital met ATS criteria for disease.

5.1.1. Risk Factors for Infection and Disease. Skin testing data from Palm Beach, Florida, reported that 32.9% of 447 participants in a population-based random household survey had a positive reaction to *M. avium* sensitin.[41] Independent predictors of a positive reaction included black race, birth outside the United States, and more than 6 years' cumulative exposure to soil. Exposure to water, food, and pets was not associated with skin test reactivity. However, the assessment of water exposure was crude and reactivity to *M. avium* sensitin may be associated with infection but not necessarily disease and thus the risk factors identified in this study may not be relevant to persons with disease.

Male sex, non-Hispanic black race, and birth outside of the United States were reported as risk factors in individuals who underwent skin testing with PPD-B as part of the National Health and Nutrition Examination Surveys (NHANES).[42] As in the previous study from Florida, occupations with the potential for extensive soil exposure, such as in the agricultural or construction industry, were more likely to be sensitized to *M. intracellulare* than persons in other occupations.

Patients who have pulmonary disease caused by NTM often have structural lung disease such as chronic obstructive pulmonary disease,[1,43] cystic fibrosis, bronchiectasis, pneumoconiosis, prior TB, alveolar proteinosis, and chronic aspiration.[44–47] In the study from France noted previously,[39] over 50% of the patients who met ATS disease criteria (except for those with *M. kansasii*) had underlying predisposing factors such as preexisting pulmonary

or immune deficiency. NTM lung disease has been associated with abnormal CF genotypes[48] and α1-antitrypsin phenotypes.[49,50] For example, among 50 patients with bronchiectasis and pulmonary NTM infection, 20% were diagnosed with cystic fibrosis and 50% of the patients had a CFTR mutation identified.[48] Among 98 patients with bronchiectasis in the United Kingdom, 10 had NTM isolated from sputum specimens and cystic fibrosis was more likely in those with NTM than without NTM.[51] In a cohort of 100 patients with rapidly growing mycobacteria, anomalous α-1 antitrypsin proteins were present in 27% of the patients, which is 1.6 times the estimated prevalence in the U.S. population.[52]

Extremely high rates of NTM disease have been reported among miners in South Africa. The incidence of NTM disease in gold miners in South Africa was reported to be 47.6 per 100,000 and that of *M. kansasii*, the most commonly isolated NTM, was 37.0 per 100,000, 34% of whom were HIV infected.[53]

Patients with abnormalities in the IFN-gamma or IL-12 pathways are predisposed to severe mycobacterial infections[54,55] as are patients receiving TNF-antagonist. HIV-infected patients who have advanced disease are at risk of developing disseminated disease. Of note, many women with NTM infection have nodular bronchiectasis and similar body types: scoliosis, pectus excavatum, mitral valve prolapse, and joint hypermobility.[56] The reason for this association has not been definitively determined.

5.2. Epidemic Behavior and Contagiousness

Most infections are believed to arise from environmental exposure to organisms in water, soil, and dust.[57–59] Person-to-person transmission and animal-to-human transmission of NTM are not important factors in acquisition of infection. Thus, community-based outbreaks are unusual although recent outbreaks of mycobacterial furunculosis associated with footbaths at nail salons have highlighted the ubiquitous nature of these organisms.[60–62] Most of the footbath-related infections have been caused by *M. fortuitum* and *M. margeritense*.

There are numerous reports of health-care-associated outbreaks of disease and pseudo-outbreaks.[63,64] Investigations of these outbreaks and pseudo-outbreaks caused by NTM have demonstrated that tap water, ice prepared from tap water, processed tap water used for dialysis, and distilled water used for preparing solutions such as gentian violet are the usual sources for organisms involved.[1]

Most health-care-associated outbreaks and pseudo-outbreaks have involved in rapid growers such as *M. fortuitum* and *M. abscessus*. Infections associated with median sternotomy wounds, injections, plastic surgery, liposuction, LASIK, dialysis-associated outbreaks, long-term central catheters, and middle ear tympanostomy tube replacement have all been associated with infections due to rapid growers.[1]

Slowly growing mycobacteria have also been implicated in several pseudo-outbreaks. For example, 65 *M. simiae* isolates from 62 patients at a community teaching hospital in Houston, Texas, were reported.[65] None of the patients were thought to have clinical evidence of disease and none were treated. The reservoir for this pseudo-outbreak was identified as a contaminated hospital water supply. A similar pseudo-outbreak was described in San Antonio, Texas.[63]

5.3. Geographic Distribution

As noted previously, studies assessing cutaneous sensitization to MAC antigens have demonstrated significant variability in sensitization across the United States. Sixty-five percent of nursing students who came from the southeastern United States had evidence of cutaneous sensitization to *M. intracellulare* antigens compared with only 29% of those from other areas of the country.[35] Skin test surveys of U.S. Navy recruits in the 1960s demonstrated higher rates of reactivity in those from the southeastern United States (56%) than those from the northern states (15–25%), suggesting higher rates of infection.[36] These surveys demonstrated the striking geographic differences in NTM infection.

There is also significant geographic variation in which NTM cause disease. *M. avium* complex has been reported as the most common cause of NTM-related pulmonary disease in almost all studies.[1] However, the next most common NTM that causes disease varies by location. For example, in the United States, *M. kansasii* is the second most common cause of pulmonary disease followed by *M. abscessus*. In Canada, and some parts of Europe, *M. xenopi* is the second most common whereas in northern Europe and Scandinavia, *M. malmoense* is second. In a recent study from France, *M. avium* complex was the most common isolate overall but *M. xenopi* was the most common isolate in Paris.[39]

5.4. Temporal Distribution

For years there has been controversy regarding whether or not the incidence of NTM is increasing. Recent data support the view that the incidence of NTM infections is increasing at least in North America (Figure 1).[40] In a retrospective cohort review from 1997 to 2003 in Ontario, Canada, 222,247 pulmonary isolates from 10,231 patients were identified. The prevalence was 9.1 per 100,000 in 1997 and increased to 14.1 per 100,000 by 2003 ($p < 0.0001$)

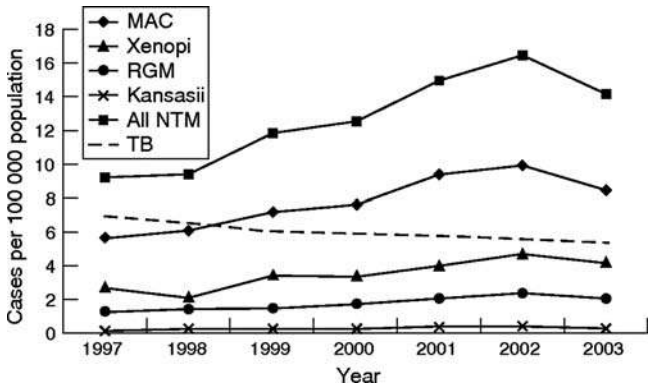


Figure 1. Prevalence of pulmonary nontuberculous mycobacteria (NTM) infections and tuberculosis (TB) case rates in Ontario 1997–2003. All NTM prevalence rates increased significantly (all $p \leq 0.001$). Tuberculosis rates calculated from total number of tuberculosis cases and population data from Statistics Canada (for 1999–2003 from http://www.statcan.ca/english/Pgdb/demo02.htm accessed October 4, 2004) and estimated by back extrapolation (for 1997 and 1998). MAC, *M. avium* complex; RGM, rapidly growing mycobacteria. From Marras et al.,[40] with permission (obtained).

with an average annual increase of 8.4%. Increases were noted among *M. avium* complex, *M. xenopi*, rapidly growing mycobacteria, and *M. kansasii*. Of note, the rate of TB declined 4.0% over the study period. Two hundred patients were evaluated in more detail and 33% fulfilled the clinical, radiological, and bacteriological ATS criteria.

In contrast, in France, a recent report describes the incidence of NTM at being low and stable over a 3-year period.[39] Data from 32 sentinel sites in France described 262 patients who met the 1997 ATS criteria for disease and reported an incidence rate of NTM disease of approximately 0.73 cases per 100,000 inhabitants. Of note, this is an underestimate of the true rate of disease since this was a laboratory-based study and many patients with NTM disease have never been suspected of having NTM disease.

The reasons for the apparent increases in NTM disease are not clear. An aging population, more immunosuppressed patients, waning cross-immunity, improved diagnostics, increased clinical suspicion, and greater environmental exposure may all play a role in the increase in disease. Data supporting the latter come from a recent study that reported on the mycobacterial sensitization to PPD-B in the United States over three decades. Using NHANES, the authors compared the frequency of skin test reactions to *M. intracellulare* antigens during two time periods, 1971–1972 and 1999–2000. Sensitization increased from 11.2 to 16.6% between the two time periods suggesting that exposure to *M. intracellulare* had increased over time. The increase was most notable in foreign-born individuals.

5.5. Age and Sex Distribution

Sensitization to mycobacterial antigens is strongly associated with age. In West Palm Beach, Florida, the prevalence of sensitization peaked in the 41- to 50-year age group[41] whereas in the study reporting sensitization to *M. intracellulare* in the NHANES surveys, the prevalence was highest in the 20- to 39-year age group.[42] In both studies, the prevalence was lower in people older than 60 years.

Age distribution of persons with NTM disease varies by mycobacterial species and site of disease.[66,67] Pulmonary disease is most commonly seen in older patients and patients with MAC lung disease tend to be older than those with *M. kansasii* disease.[68] Patients with lung disease due to rapidly growing mycobacteria are typically at least 60 years of age.[69] In patients with nonpulmonary infections, the age spectrum is greater.

Two studies have reported that sensitization to *M. avium* and *M. intracellulare* antigens is more common among men than women.[41,42] This increase in sensitization is likely related to greater environmental exposure in men than women. Male predominance is marked among persons with pulmonary *M. kansasii* and less so among patients with *M. avium*.[68] However, among older persons with *M. avium* complex pulmonary disease and those with nodular bronchiectatic disease, women predominate.[39] There is a significant female predominance among those with lung disease due to rapidly growing mycobacteria.[69]

6. Mechanisms and Routes of Transmission

6.1. Nontuberculous Mycobacteria in the Environment

The source of NTM infection appears to be through environmental exposure and transmission from human to human has not been described. Molecular epidemiology studies of *M. abscessus* in patients with cystic fibrosis have demonstrated that infections appear to be acquired and not related to person-to-person transmission.[45,70,71] In contrast, genotyping methods have demonstrated similar patterns between isolates cultured from patients and their environments.[72–74] In most cases, the source of infection has been water although soil has also been implicated.[73] A recent study from Japan evaluated the water from the residences of 49 patients with MAC and 43 healthy volunteers. MAC was isolated from 11 samples from multiple bathroom samples but from not other sites in the home. All but one sample came from the patient's homes. In two patients the PFE and RFLP patterns were the same.[75]

NTM have been found in municipal water supplies or treated water supplies from 95 of 115 (83%) dialysis

centers throughout the United States.[76] MAC isolates were recovered from 42 of 108 (32%) of tested locations such as homes, hospitals, commercial buildings, and reservoirs in Los Angles, California.[1] In a study of 50 biofilm samples from a variety of pipe systems in Germany, 90% of the samples contained NTM.[77] At Grady Memorial Hospital in Atlanta, hospital water was felt to be the source of some infections; 25% of patients with MAC disease had the same pulse field pattern as that from hospital water.[78]

7. Pathogenesis and Immunity

The importance of host immunity has been demonstrated in individuals and families with mutations in the IFN-gamma/IL-12 pathway genes[54,55,79] in whom disseminated mycobacterial infections have been reported. In in vitro studies, α-1 antitrypsin synthetic inhibitor of serine proteases, was reported to suppress *M. abscessus* infection in monocyte-derived macrophages by up to 65% ($p < 0.01$), suggesting that α-1 antitrypsin may play a role as an anti-rapid grower host defense factor.[52]

In a study from Japan, 170 patients with MAC lung infection were studied, and of 622 siblings of these patients, 3 had MAC lung disease also.[80,81] The authors concluded that the rate of infection among the siblings was higher than previously estimated among the general population and thus suggested a genetic predisposition to disease. Despite these types of studies, there is little evidence suggesting a host-immune defect in most patients with pulmonary infection.

In patients with HIV infection, disseminated NTM infections typically occur only after the CD4 lymphocyte count falls below 50 cells/μl suggesting that specific T-cell products or activities are required for mycobacterial resistance.[1] MAC presumably enters the body through ingestion of contaminated food or liquid and subsequently spreads through the bloodstream.

There is a striking association between bronchiectasis, nodular pulmonary NTM infections, and a particular body habitus, predominantly in postmenopausal women.[56] It remains to be seen whether or not these women have some sort of subtle immune deficiency that predisposes them to NTM pulmonary infections or whether their predisposition is related to ineffective mucociliary clearance or poor tracheobronchial secretion drainage.

Unlike *M. tuberculosis*, the NTM do not appear to live in a state of dormancy. Moreover, simply isolating NTM from a respiratory specimen does not mean that the patient has NTM-related disease. For years the term "colonization" has been used to differentiate those who have a single or small number of positive cultures over time and in whom progressive disease cannot be demonstrated.[81] At least two observations suggest that colonization is uncommon. First, Prince and colleagues described 21 patients with pulmonary MAC who had no underlying lung disease, most of whom had noncavitary disease that was slowly progressive.[82] Subsequently, Erasmus and colleagues reported the findings on CT demonstrating multiple small nodules and associated cylindrical bronchiectasis in these patients.[83] Taken together, these studies suggested that some patients who were thought to have been colonized did in fact have evidence of disease on CT scans and demonstrated slow clinical and radiographic progression.

8. Patterns of Host Response

8.1. Clinical Features

The most common clinical manifestation of NTM infection is chronic lung disease.[1] However, lymphatic, skin/soft tissue, bone/joint involvement as well as disseminated disease are also important manifestations of infection. The propensity of a specific manifestation varies with the specific species and certain host factors. For example, the rapid growers, while a common cause of lung disease, have a propensity for producing skin and soft tissue infections. HIV-infected patients typically present with disseminated disease whereas persons with cystic fibrosis and elderly white females present with pulmonary disease.

8.1.1. Pulmonary Disease. Chronic pulmonary disease is the most common clinical presentation of NTM disease and usually presents with chronic cough, fatigue, fever, hemoptysis, chest pain, and weight loss. Physical examination may identify certain morphologic characteristics in postmenopausal women and, occasionally men, that include thin body habitus, scoliosis, pectus excavatum, and mitral valve prolapse.

The radiographic features in patients with MAC pulmonary disease are typically divided into primarily nodular bronchiectatic or fibrocavitary disease. In patients with fibrocavitary disease, the radiographic abnormalities consist of upper lobe opacities with evidence of cavitation and volume loss, all suggestive of tuberculosis (Figure 2a, b). Patients with predominantly noncavitary nodular bronchiectatic disease have opacities in the mid and lower lung fields (Figure 3a). High-resolution computed tomography scans demonstrate bronchiectasis often in the middle lobe and lingula with evidence of centrilobular nodules referred to as a "tree-in-bud" pattern (Figure 3b).

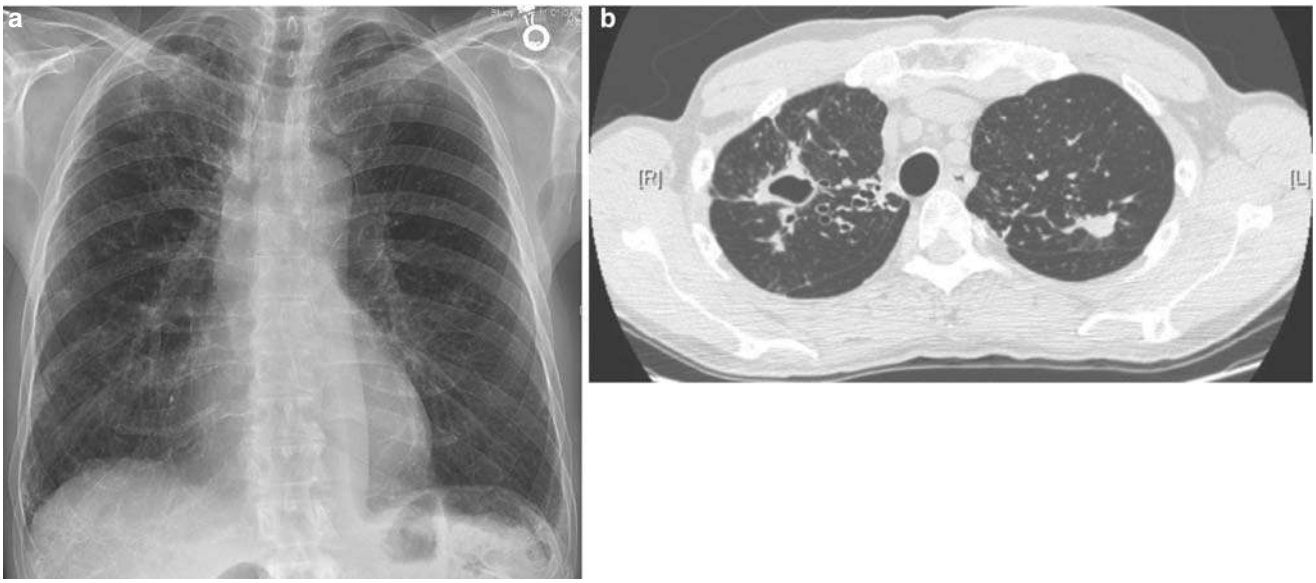


Figure 2. A 76-year-old man with pulmonary *M. intracellulare* disease. (**a**) The chest radiograph demonstrates bilateral upper lobe opacities with a right upper lobe cavity. (**b**) A high-resolution chest computed tomogram demonstrates a right upper lobe cavity with surrounding bronchiectasis and nodules as well as a left upper lobe nodular opacity.

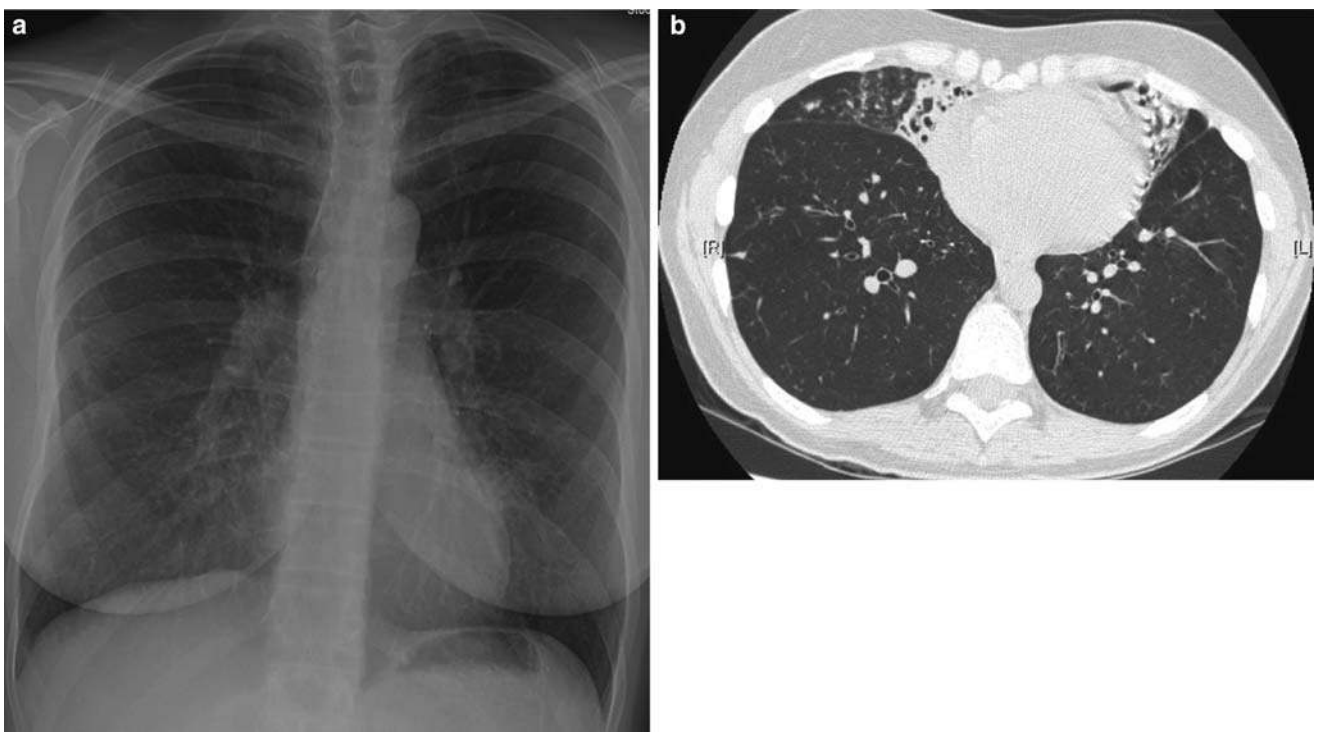


Figure 3. A 50-year-old woman with pulmonary *M. intracellulare* disease. (**a**) The chest radiograph demonstrates right middle lobe and lingula opacities. (**b**) A high-resolution computed tomogram demonstrates right middle lobe and lingular bronchiectasis with some consolidation and centrilobular nodules.

Patients with *M. kansasii* infection usually present with upper lobe cavitary opacities and the cavities are typically thin walled. The chest radiograph in patients with rapidly growing mycobacterial infections usually shows multilobar, patchy, reticulonodular, or mixed interstitial alveolar opacities with an upper lobe predominance. High-resolution CT will show bronchiectasis and small nodules similar to MAC (Figure 4a, b).

M. xenopi is second to MAC as a cause of pulmonary disease in Canada and parts of Europe. Typically, patients with disease due to *M. xenopi* present with underlying chronic obstructive pulmonary disease and the radiographic appearance is similar to that seen in upper lobe cavitary MAC disease.

8.1.2. Lymphadenitis. The most common form of NTM disease in children is cervical lymphadenitis.[1] *M. avium* is the most common etiology accounting for 80% of culture-proven cases. *M. scrofulaceum* is the second most common cause of lymphadenitis in the United States and Australia whereas *M. malmoense and M. haemophilum* are more common in Scandinavia, the United Kingdom, and other areas of northern Europe. *M. tuberculosis* is only isolated in approximately 10% of culture-proven mycobacterial cervical lymphadenitis in the United States but in adults 90% are due to TB.[1]

Infection usually involves the submandibular, submaxillary, cervical, and preauricular lymph nodes in children between 1 and 5 years of age. Involvement of the lymph nodes is usually unilateral and nontender. The lymph nodes may enlarge and eventually rupture producing sinus tracts just like tuberculous lymphadenitis.

8.1.3. Soft Tissue, Skin, and Bone Infection. Although virtually any NTM can cause skin, soft tissue, and bony infection, the most common species to do so are the rapid growers.[84,85] Rapid growers often produce infections at the site of punctures or surgery and present with tender subcutaneous nodules.

8.1.4. Disseminated Infections. Disseminated infections are most commonly associated with advanced HIV infection and other forms of severe immunosuppression. Over 90% of reported disseminated infection in HIV-infected patients are due to MAC and almost all of these are due to *M. avium*.[1] The next most common cause of disseminated NTM disease in HIV patients is *M. kansasii* but a number of other species have also been implicated. Patients present with advanced HIV disease and complain of fever, night sweats, abdominal pain, diarrhea, and weight loss. In non-HIV-infected patients, fever of unknown origin is a common presentation.

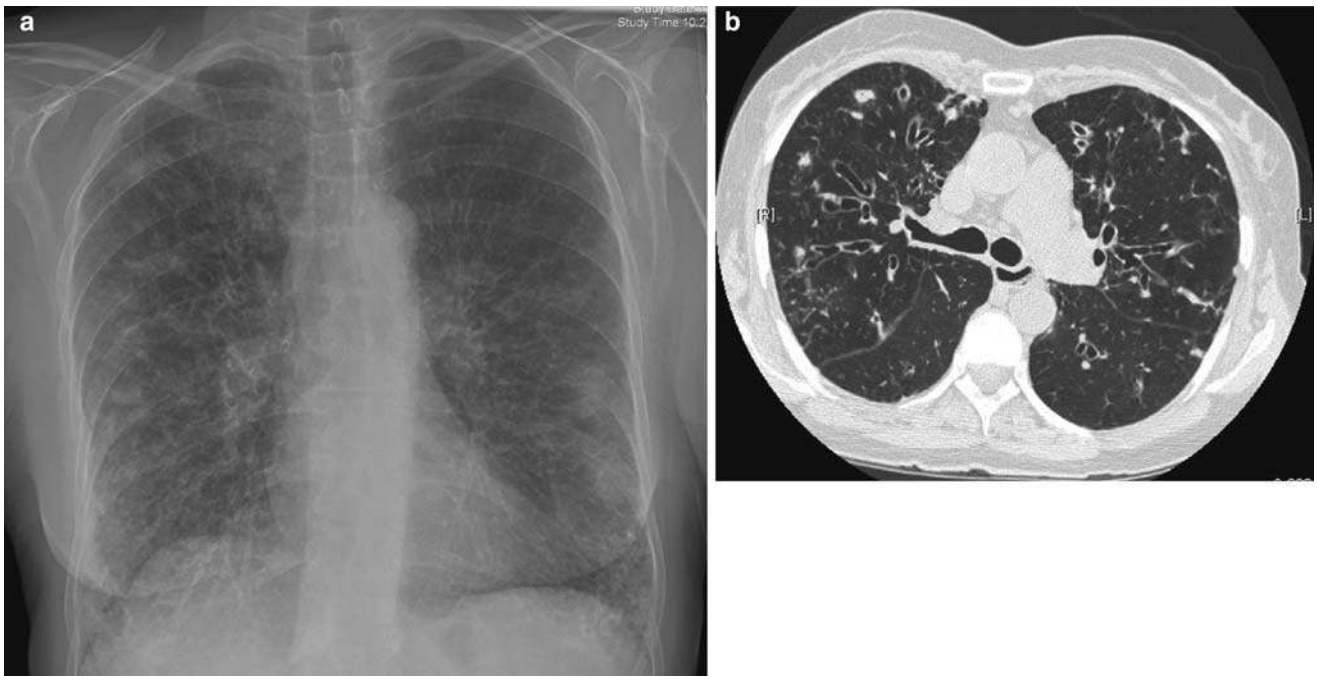


Figure 4. An 84-year-old woman with a history of pulmonary *M. intracellulare* infection who now presents with pulmonary *M. abscessus* infection. (**a**) The chest radiograph demonstrates extensive bronchiectasis with diffuse nodules and airspace opacities. (**b**) A chest computed tomogram demonstrates extensive bronchiectasis with numerous nodules.

8.2. Diagnosis

Finding mycobacteria in a patient's specimen leads to the question of whether the isolate is causing infection/disease in the patient or is simply a contaminate or colonizer. To exclude the transient occurrence of NTM in the patient's respiratory specimen due to exposure to the bacteria, aerosolized from waters or soil, specific criteria have been recommended for the diagnosis of pulmonary disease caused by NTM (see below).[1,86]

8.2.1. Pulmonary Disease. Patients suspected of having a pulmonary NTM infection should be evaluated with a chest radiograph and/or high-resolution computed tomography, particularly when there is no evidence of cavitation on the radiograph. If there is evidence of radiographic abnormalities consistent with an NTM infection, at least three sputum specimens should be obtained for AFB examination and mycobacterial culture. TB as well as other disorders should be excluded.

The clinician must weigh clinical, bacteriologic, and radiographic information in order to diagnose pulmonary NTM infection (Table 2).[1] NTM pulmonary infections should be suspected when a patient presents with a compatible clinical picture and has nodular or cavitary opacities on the chest radiograph or an HRCT scan that shows multifocal bronchiectasis with multiple small nodules. In addition to these clinical criteria, the patient should have at least two positive cultures from separate sputum specimens or a positive culture from at least one bronchial wash or lavage. Ninety-eight percent of patients with two or more positive sputum cultures progressed without treatment in a study published from Japan.[87] Additional diagnostic criteria include transbronchial or other lung biopsy with mycobacterial histopathological features and positive culture for NTM or biopsy showing mycobacterial histopathologic features and one or more sputum or bronchial washings that are culture positive. Some patients who do not meet these criteria at the initial evaluation will do so later so close follow-up is recommended.

8.2.2. Lymphadenitis. Diagnosis is usually made by fine needle aspiration or surgical excision of the involved lymph nodes. However, only 50–82% of excised nodes will be culture positive.[1]

8.2.3. Soft Tissue. The most sensitive way to diagnose infections due to rapid growers is to obtain a tissue biopsy that is cultured and examined histopathologically. Unfortunately, visualization of mycobacteria in the specimen is seen in only about 10% of cases and the yield of culture is not known since most cases that have been reported were culture confirmed.[88,89]

8.2.4. Disseminated Disease. Diagnosis is usually through detection of the causative organism in the blood. In HIV-infected patients *M. avium* can be isolated in over 90% of cases.[1]

9. Control and Prevention of NTM Infections

9.1. General Concepts

There are several differences regarding the treatment of tuberculosis and that of infections caused by NTM. First, not all patients who have an NTM isolated from their sputum specimen require treatment. As noted previously, in some instances, individuals will grow an NTM species from their respiratory specimens but they are both asymptomatic and demonstrate no radiographic progression. Second, unlike with *M. tuberculosis*, in vitro susceptibility results for many NTM do not correlate well with clinical response to antimicrobial therapy; thus clinicians should use such data with a clear understanding of the limitations. Third, while most patients with tuberculosis can be treated with a single four-drug regimen, the many species of NTM dictate

Table 2. Clinical, Radiographic, and Microbiologic Criteria for Diagnosing Nontuberculous Mycobacterial Lung Disease

Clinical	Compatible pulmonary symptoms *and*
Radiographic	Nodular or cavitary opacities on chest radiograph or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules *and* Appropriate exclusion of other diagnoses *and*
Microbiologic	Positive culture results from at least two separate expectorated sputum samples if results are nondiagnostic, consider repeat sputum AFB smears and cultures *or* Positive culture result from at least one bronchial wash or lavage *or* Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM

Adapted from Griffith et al.[1]

Table 3. Drugs and Dosages Used to Treat Nontuberculous Mycobacterial Infections in Adults

Drug	Daily dose (max)	Three times/week dose (max)
Oral drugs		
Isoniazid	5 mg/kg/day (300 mg)	15 mg/kg/day (900 mg)
Rifampin	10 mg/kg/day (600 mg)	10 mg/kg/day (600 mg)
Rifabutin	5 mg/kg/day (300 mg)	5 mg/kg/day (300 mg)
Ethambutol	15–20 mg/kg/day (1.6 g)	20–35 mg/kg/day (2.5)
Azithromycin	250 mg/day	500–600 mg/day
Clarithromycin	500–1,000 mg/day	1,000 mg/day
Doxycycline	100 mg/day (100 mg)	–
Trimethoprim–sulfamethoxazole	160 mg trimethoprim/800 mg sulfamethoxazole twice daily	–
Ciprofloxacin	500–750 mg twice daily	–
Moxifloxacin	400 mg/day (400 mg)	–
Linezolid	600 mg once or twice daily	–
Parenteral drugs		
Tobramycin	4–7 mg/kg/day IV	–
Streptomycin	10–15 mg/kg/day (1 g) IV or IM	15–20 mg/kg (1.5) IV or IM
Amikacin/kanamycin	10–15 mg/kg/day (1 g) IV or IM	15–20 mg/kg (1.5) IV or IM
Cefoxitin	100–200 mg/kg (12 g/day) in divided doses IV	–
Imipenem	500–1,000 mg 2–3 times/day IV	–
Tigecycline	50 mg twice daily IV	–

IV – intravenously, IM – intramuscularly.

different drugs and regimens (Table 3). Fourth, NTM infections usually require a longer duration of therapy than with tuberculosis, often up to 2 years. And finally, surgical resection/excision is used more frequently in the management of NTM infection than with tuberculosis. As with tuberculosis, NTM should be treated with more than one antimycobacterial drug in order to prevent the emergence of resistance.

The role of in vitro susceptibility testing for managing patients with NTM disease remains controversial. In vitro susceptibility results and clinical outcome do not always correlate and this is particularly true of infections due to *M. avium complex, M. xenopi,* and *M. simiae*.[1] On the other hand, there appears to be better correlation with *M. kansasii* and the rapid growers.

9.2. Chemotherapy

9.2.1. Chemotherapy of Pulmonary Infections.

9.2.1.1. Mycobacterium avium Complex. Prior to the availability of the newer macrolides, the long-term treatment outcomes for patients treated with antituberculosis regimens were approximately 50%.[1] Small, noncomparative studies of azithromycin- and clarithromycin-containing regimens have been associated with higher bacteriologic response rates but long-term follow-up is often unavailable. Treatment responses are also variable with cure rates ranging from 60 to 85%.[90–96]

The ATS currently recommends that the treatment regimen be based on the presence or absence of cavitary disease and whether or not the patient has been treated previously (Table 4).[1] A three-times-a-week regimen is recommended for patients with noncavitary nodular bronchiectasis. A recent study reported that the bacteriologic response to three-times-a-week therapy was poor overall.[95] However, the response rate was better in those with noncavitary disease compared with those who had cavitary disease (71 versus 20%). Factors associated with a good bacteriologic response were having noncavitary disease, being AFB smear negative, no previous treatment for MAC lung disease, older age, and longer duration of ethambutol use. Eight of 139 patients receiving daily ethambutol therapy developed optic neuritis compared with 0 of 90 on intermittent therapy; thus, three-times-a-week dosing may be associated with less optic neuritis than daily dosing.[97]

Daily therapy is recommended for patients with cavitary and/or advanced disease and in those who have been treated previously.[1] An aminoglycoside should be considered in these patients, at least for the first 2–3 months of therapy although a recent study suggested that there was no long-term benefit to this practice.[96] Surgery should be considered in patients who have a macrolide-resistant strain of MAC, have failed treatment, or have primarily focal cavitary disease. Surgery should be performed by a surgical team that is experienced in managing these difficult cases.[98–102]

Table 4. Treatment of Pulmonary Disease Due to *M. avium* Complex Infections

Type of disease	Regimen
Nodular/bronchiectatic disease	Clarithromycin 1,000 mg tiw or azithromycin 500–600 mg tiw and Ethambutol 25 mg/kg tiw and Rifampin 600 mg tiw
Cavitary disease	Clarithromycin 500–1,000 mg/day or azithromycin 250–300 mg/day and Ethambutol 15 mg/kg daily and Rifampin 450–600 mg daily and Streptomycin or amikacin 15 mg/kg tiw
Advance or previously treated	Clarithromycin 500–1,000 mg/day or azithromycin 250–300 mg/day and Ethambutol 15 mg/kg daily and Rifabutin 150–300 mg daily or rifampin 450–600 mg daily and Streptomycin or amikacin 15 mg/kg tiw

Adapted from Griffith et al.[1]
tiw – three times per week.

9.2.1.2. M. kansasii. Patients who are treated with the currently recommended treatment regimen respond well and both treatment failure and relapse are uncommon. Among 180 patients in three studies, the 4-month culture conversion rates were 100% and there was only one long-term relapse.[103–105] Patients with lung disease due to *M. kansasii* should be treated with isoniazid, rifampin, and ethambutol[1] although a regimen including clarithromycin, rifampin, and ethambutol has also been used successfully.[106] As with other pulmonary NTM infections, the treatment duration should include 12 months of negative sputum cultures. For patients whose isolate is resistant to the rifamycins, a three-drug regimen is recommended based on in vitro susceptibility data to clarithromycin or azithromcyin, moxifloxacin, sulfamethoxazole, or streptomycin.[1] Surgical resection is seldom necessary in patients infected with *M. kansasii*.

9.2.1.3. Mycobacterium xenopi. The optimal treatment regimen and duration of therapy for pulmonary disease due to *M. xenopi* are not known, and in vitro susceptibility results may be difficult to interpret because the response of the organism does not always correlate. Patients are usually treated with a typical anti-MAC regimen that includes a newer macrolide, ethambutol, and rifampin, and responses may be enhanced by addition of a newer fluoroquinolone.[1] The mortality rate with *M. xenopi* infection has been reported to be as high as 57%, but this probably reflects the severity of the underlying lung disease.[107]

9.2.1.4. Rapidly Growing Mycobacteria. Treatment outcomes with *M. abscessus* are generally poor, with no antibiotic regimen able to provide long-term sputum conversion in patients with pulmonary disease. *M. abscessus* is susceptible to only a few antimicrobials including the macrolides, imipenem, cefoxitin, amikacin, tigecycline, and occasionally linezolid. Therefore, in vitro susceptibility testing is recommended for selection of a treatment regimen.[1] Current recommendations are to provide periodic drug administration of multidrug therapy including a macrolide, and one or more parenteral agents such as amikacin, cefoxitin, or imipenem for 4–6 months to help control symptoms and prevent progression.[1] Surgical resection should be considered for select patients but only after a period of intensive antimicrobial therapy and only if performed by an experienced surgeon.

M. chelonae is usually susceptible to tobramycin, macrolides, linezolid, imipenem, and amikacin and may demonstrate susceptibility to fluoroquinolones and doxycycline.[1] Isolates are usually resistant to cefoxitin. Treatment should consist of at least two drugs to which there has been demonstrated in vitro drug susceptibility and the duration should be for at least 12 months of culture negativity.

M. fortuitum isolates are usually susceptible to newer macrolides, flouroquinolones, doxycycline, minocycline, sulfonamides, cefoxitin, and imipenem.[1] Therapy should consist of at least two agents with in vitro activity for at least 12 months of culture negativity.

9.2.2. Chemotherapy of Lymphadenitis. Cervical lymphadenitis is usually caused by *M. avium* complex or *M. scrofulaceum* infection.[1] Surgical excision of the involved lymph nodes without chemotherapy is usually curative with success rates of approximately 95%. In patients who fail surgical excision a macrolide-based treatment regimen with or without repeat excision is usually successful.

9.2.3. Chemotherapy of Skin, Soft Tissue, and Bone/Joint Infections. A combination of surgical excision plus multidrug chemotherapy is usually successful for treatment of MAC infections of the skin and soft tissues. The optimal duration of therapy is not known but should probably be 6–12 months in duration.[1] For serious infections caused by rapidly growing mycobacteria, a newer macrolide should be combined with a parenteral medication (amikacin, cefoxitin,

or imipenem) or perhaps another oral agent in the case of infection due to *M. fortuitum*.[1] Therapy should continue for a minimum of 4 months for skin infections and at least 6 months for bone infections. Surgery is generally indicated for extensive disease, and removal of foreign objects such as breast implants, percutaneous catheters, and joint prostheses is necessary.

9.2.4. Chemotherapy for Disseminated Infections. Treatment of disseminated MAC infection in HIV-infected patients should include clarithromycin 500 mg twice daily or azithromycin 500 mg daily, ethambutol 15 mg/kg daily with or without rifabutin 300 mg daily.[1] Treatment should be considered lifelong unless immune restoration is achieved by antiretroviral therapy. MAC treatment can be stopped for patients who are asymptomatic and have achieved a CD4 lymphocyte count of over 100 cells/μl for at least 12 months.[108]

9.3. Prophylaxis of NTM Infections

Prophylactic therapy for NTM is only recommended in the setting of advanced HIV disease. Preventive therapy for disseminated MAC is recommended for HIV-infected patients with fewer than 50 CD4 lymphocytes/μl.[108] Azithromcyin given as 1,200 mg once weekly is the preferred agent but alternative regimens include clarithromcyin 500 mg twice daily or rifabutin 300 mg daily.[109] Primary prophylaxis can be discontinued when patients have responded to antiretroviral therapy with an increase in CD4 cell count to more than 100 cells/μl for more than 3 months.[108,110] Prophylaxis should be reintroduced if the cell count falls to less than 50–100 cells/μl.

As noted previously, NTM infections in health-care settings have been associated with contamination of water sources, biologicals, and multidose vials used in postsurgical infections.[1] In these settings, specific steps can be taken to in order to prevent the use of tap water to wash wounds or equipment and avoidance of multidose vials should be avoided for injections.

Prevention of community-acquired pulmonary infections remains difficult. The organisms have been isolated in tap water and water distribution systems, may tolerate high water temperatures, and are resistant to our typical decontamination methods.[1] Therefore, it is not clear how to best decrease our environmental exposure to these hardy organisms.

9.4. Immunization

No vaccine has been developed for treatment or prevention of NTM infections. However, recent reports suggest that the tuberculosis vaccine Bacille Calmille Guerin (BCG) has some protection against NTM infection. In Sweden, NTM lymphadenitis in children increased after mass BCG vaccination was discontinued in 1975.[111,112] Among the cohorts born in Sweden in the period 1975–1985, the cumulative incidence rate before 5 years of age was estimated at 26.8 per 100,000 in non-BCG-vaccinated children and at 4.6 among those vaccinated.[112] Similarly, in the Czech Republic, *M. avium* lymphadenitis was more common in children who had not received BCG vaccine compared with vaccinated children.[113]

10. Unresolved Problems

The decline of tuberculosis in many countries has been associated with an increase in the number of NTM infections. The reasons for this and just how much NTM disease exists are not known because of the lack of epidemiologic and surveillance data. In order for us to better understand these infections, we need better data on the natural history of NTM infections and how and where individuals become infected. Better diagnostic tests are needed to help us differentiate diseased from non-diseased individuals and we need tests that will correlate with clinical outcomes. Except for pulmonary disease due to *M. kansasii*, the clinical outcomes with most pulmonary NTM infections are suboptimal, so new drugs and better treatment regimens are greatly needed. It is remarkable how little we know about these ubiquitous organisms and the infections they produce. Only better epidemiologic data and clinical trials can answer these many questions.

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CHAPTER 42

Tularemia

Paul S. Mead and Jeannine M. Petersen

1. Introduction

Tularemia is an uncommon disease caused by *Francisella tularensis,* a highly infectious gram-negative coccobacillus found in rabbits, hares, rodents, and arthropods throughout the northern hemisphere. Humans become infected through arthropod bites, handling infected animal tissues, ingestion of contaminated food or water, and inhalation of contaminated aerosols. Symptoms vary according to the route of inoculation. The most common clinical syndrome is ulceroglandular tularemia, which is characterized by fever, a skin ulcer at the site of inoculation, and regional lymphadenitis. Other syndromes include glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal tularemia. Two subspecies, *F. tularensis* ssp. *tularensis* (type A) and *F. tularensis* ssp. *holarctica*(type B), cause the majority of human infections. Because of its ability to cause disease by aerosol, *F. tularensis* has been developed by several countries as a potential bioweapon.

2. Historical Background

Tularemia-like illness (Yato-byo) was recognized as early as 1818 in Japan, where court physician, Homma Soken attributed the illness to the ingestion of hare meat.[1] In 1911, McCoy and Chapin isolated the etiologic agent while looking for plague in California ground squirrels. They named the organism *Bacterium tularense* for Tulare County where the infected squirrel was collected, and they demonstrated passage to rabbits, mice, guinea pigs, and monkeys.[2] Three years later, Wherry, Lamb,[3] and Vail[4] isolated *B. tularense* from the eye of a 28-year-old Ohio butcher with conjunctivitis, fever, and regional lymphadenopathy. The butcher's illness was attributed to handling meat of local wild rabbits, which had been dying mysteriously in large numbers.[5] In 1919, Francis isolated *B. tularense* from the blood of patients with "deer fly fever" in Utah and coined the term "tularemia" to describe the associated illness. In 1924, Ohara independently described a febrile illness associated with butchering hares and replicated the illness by rubbing hearts from ill hares onto the hand of his wife, who had volunteered for the experiment. Two years later, Ohara recognized the similarities between this illness and tularemia and sent specimens to Francis who confirmed it as due to *B. tularense.*[6] Between 1928 and 1935, cases of tularemia began to be reported in Russia, Norway, Canada, Sweden, and Austria.[7–9]

In 1959, the organism was placed in it own genus, *Francisella*, based on morphology, culture, biochemical, and pathogenic properties.[10] That same year, Olsufiev concluded that there were two distinct types of tularemia, with differences in virulence, geographic distribution, animal reservoirs, and culture characteristics of the causative organisms.[11–13] The names *Francisella tularensis tularensis* and *Francisella tularensis palaearctica* (*holarctica*) were proposed for these two subspecies.[10]

As understanding of the ability of *F. tularensis* to cause epidemics, epizootics, and laboratory infection increased, the potential of *F. tularensis* as a bioweapon was explored. Experiments with human volunteers in 1961 confirmed the potential for intentional airborne transmission.[14] Tularemia was subsequently developed as a biological weapon by the Soviet Union, the United States, and possibly other countries.[13] In more recent years, concern about bioterrorism has lead to renewed research in tularemia.[15]

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3. Methodology

Current understanding of tularemia is based on case reports, outbreak investigations, routine surveillance, ecological studies, and laboratory experiments conducted on both animals and humans. Recent advances in molecular techniques have yielded improved diagnostic tests and a better understanding of the organism's genetic diversity and pathogenic properties.

3.1. Sources of Data

Global statistics on tularemia incidence in humans are not available. In the United States, tularemia has been a reportable condition since 1927, with a brief hiatus from 1995 to 1997.[16] State and local health departments report cases electronically to the Centers for Disease Control and Prevention (CDC), which publishes provisional and final tallies in the *Morbidity and Mortality Weekly Report* (MMWR). Surveillance summaries for various time periods are also available for Sweden,[17] Japan,[18] Slovakia,[19] and Turkey.[20] Under-reporting is likely given the bacterium's fastidious nature and the self-limiting course of many infections.

3.2. Surveys

Past infection with *F. tularensis* can be measured by serology or a skin test,[21] which though sensitive is no longer available. Surveys for human infection have generally focused on populations thought to be at greater than average risk, including Native Alaskans,[22] animal trappers,[23–25] farmers,[26,27] workers in sugar beet factories,[28] and landscapers.[29] Although not representative of the general population, these surveys have demonstrated high rates of seropositivity in some populations and the potential for mild and asymptomatic infection.

4. Biological Characteristics

4.1. Bacteriology

F. tularensis is a small (0.2×0.2–$0.7\ \mu m$), weakly staining, gram-negative, pleomorphic coccobacillus. Members of the *Francisella* genus are characterized by a unique fatty acid content and are not phylogenetically related to any known human pathogen. *Francisella* are strictly aerobic and non-spore forming.[30]

On the basis of DNA hybridization, three species have been identified within the genus *Francisella*, *F. tularensis, F. novicida*, and *F. philomiragia*. *F. philomiragia* is a rare cause of illness among immune compromised persons and victims of near-drowning.[31–33] It can be distinguished from *F. tularensis* by biochemical reactions, most practically a positive oxidase reaction. First discovered near the Great Salt Lake in Utah, *F. philomiragia* is capable of growing in nutrient broth containing 6% NaCl and is often associated with brackish habitats. In spite of these phenotypic differences, the two species show >98.3% similarity by 16S rRNA sequencing.[30]

F. novicida is a similarly rare cause of human illness, seen primarily in immunocompromised patients; it has not been associated with any animal species or arthropod vectors.[33,34] Also isolated from the Great Salt Lake in Utah, *F. novicida* is capable of growing in nutrient broth containing 6% NaCl. A wound infection with *F. novicida* in an Australian fish farmer represents the sole human *Francisella* isolate from the southern hemisphere.[34] *F. novicida* shows >99.8 similarity to *F. tularensis* by 16S rRNA sequence comparison and has been proposed reassignment as a subspecies of *F. tularensis*.[35]

Within the species *F. tularensis*, three subspecies are currently recognized: ssp. *tularensis* (type A), ssp. *holarctica* (type B), and ssp. *mediasiatica*. The subspecies share >99.8% similarity by 16S rRNA sequencing.[36] Subspecies *tularensis* (type A) and subspecies *holoarctica* (type B) account for nearly all human infections. With the exception of a recent collection of isolates recovered near Bratislava in Slovakia,[37] type A strains are found only in North America. Type B strains are found throughout the northern hemisphere in Europe, Asia and North America. Type A strains have been considered more virulent than type B strains; however, recent studies suggest that the reverse may be true for type A strains from the western United States.[38] Subspecies *mediasiatica* has been isolated from animals in central Asia but has not been reported as a cause of human illness. Virulence on *Ft mediasiatica*, based on animal studies, is thought to be moderate.

Recent studies suggest that additional, unnamed species likely exist in the environment. For example, PCR amplification of DNA extracts from soil and water samples from the Houston area found evidence of a wide variety of *Francisella*-related organisms, including sequences that were 99.9% similar to previously reported sequences from *F. novicida* isolates.[35]

4.2. Ecology

The ecology of *F. tularensis* is complex and poorly understood. In nature, *F. tularensis* subspecies type A and type B are thought to be maintained in distinct, but incompletely defined cycles, with type A occurring in ter-

restrial environments and type B associated more often with aquatic environments. In the terrestrial cycle, rabbits and hares typically serve as amplifying hosts and ticks or biting flies as arthropod vectors. In the aquatic cycle, beaver, muskrat, and voles serve as important mammalian hosts and appear to shed live organisms into their environments. Recent studies have shown that type B *F. tularensis* can also infect free living amoeba in a manner reminiscent of *Legionella*, suggesting a role for amoeba as a natural reservoir[39] and potentially explaining the association with aquatic environments.[40]

F. tularensis can infect over 100 animal species, although not all become ill.[41] The organism has been recovered from a wide variety of vertebrates, including domestic mammals (cats, sheep), birds (grouse, pheasants, quail, mallards), reptiles, amphibians, and fish. The animals considered most important in enzootic cycles are rabbits, hares, and rodents, especially voles, muskrats, and beavers. The importance of any particular species as a reservoir or source of infection may vary depending on geographic location and local ecological factors. In North America, cottontail rabbits (*Sylvilagus*) and jackrabbits (*Lepus* spp.) are both highly susceptible to disease and linked to human infection. In Europe, hares (*Lepus* spp.) and voles (*Arvicola terrestris*) are highly susceptible to disease and linked to human infection.

A number of different arthropods have also been found to be infected with *F. tularensis*. Ticks are believed to play an important role both as vectors transmitting *F. tularensis* to vertebrates[7] and as reservoirs that maintain the organism over time through transstadial passage.[41,42] Early investigators also reported transovarian transmission; however, this has not been confirmed in later studies.[42] In North America, natural infection has been described in at least 13 species of ticks belonging to four genera, *Dermacentor*, *Ixodes*, *Amblyomma,* and *Haemaphysalis*.Transmission of tularemia to humans has been associated in particular with the *D. andersoni* (the wood tick), *D. variabilis* (the American dog tick), *D. occidentalis* (the pacific coast dog tick), and *Amblyomma americanum* (the lonestar tick). The rabbit tick, *Haemaphysalis leporis-palustris*, is thought to play a key role in maintaining the enzootic cycles in rabbits and may serve as a bridging vector for transmission to birds such as grouse, on which they also feed.[42] In Eurasia, *F. tularensis* has been isolated from *I. ricinus, D. reticulatus*, *D. marginatus*, *H. concinna, Rhipicephalus rossicus*, and a variety of other species in these genera.[12,19,12,43,44] Although *Ornithodorus* ticks may be capable of transmitting *F. tularensis* experimentally, there is no evidence that argasid ticks are important vectors in nature.[41]

Tabanid flies, including *Chrysops discalis*and *C. fulvaster*, have been found naturally infected in North America and Eurasia. These biting flies are mechanical vectors that can transmit infection to a wide a range of hosts, including humans. *F. tularensis* has reportedly been isolated from various species of mosquitoes in Scandinavia and Russia, with mechanical transmission to humans reported. Natural infection of mites and fleas has been reported, but these taxa are generally considered of minor importance in disease maintenance or transmission.[42]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Over the last century, tularemia has become a rare disease in the United States. Reported cases have declined from over 1,600 cases in 1940 to 154 cases in 2005 (Figure 1), yielding a current national incidence of 0.05 cases per 100,000 population per year. This decrease has been attributed to a marked decline in rabbit hunting as a source of sustenance and entertainment.[41] Although tularemia has been reported from all states except Hawaii, just four states accounted for 56% of all cases reported in the United States from 1990 to 2000.[45] Population-based incidence rates for Europe and Asia are not readily available and may not be particularly informative given the wide annual variation in reported cases in some regions.

5.2. Epidemic Behavior

Epidemics and outbreaks of human tularemia usually occur in association with animal epizootics, although these may not be recognized without a careful environmental investigation. In Sweden, quiescent periods with few or no reported cases are punctuated by epidemic years with hundreds or even thousands of human cases.[17,46,17,47] In contrast, the number of reported cases is reasonably consistent from year to year in the United States, although local outbreaks and clusters do occur. Epidemics among humans may also be triggered by war and social disruption.[47] Large outbreaks were observed in eastern Europe and the Soviet Union during World War II,[12] in Japan during postwar reconstruction,[48] and more recently in Kosovo following several years of civil unrest.[49]

As with other diseases, the study of tularemia outbreaks has done much to illuminate sources and mechanisms of transmission. Following on the work of Francis, deer flies (*C. discalis*) have been implicated in several outbreaks of human tularemia, all in the western United States.[50–52] In a 1973 outbreak in Utah, 73% of 38 patients reported a definite or likely deer fly bite.[51] A field investigation confirmed

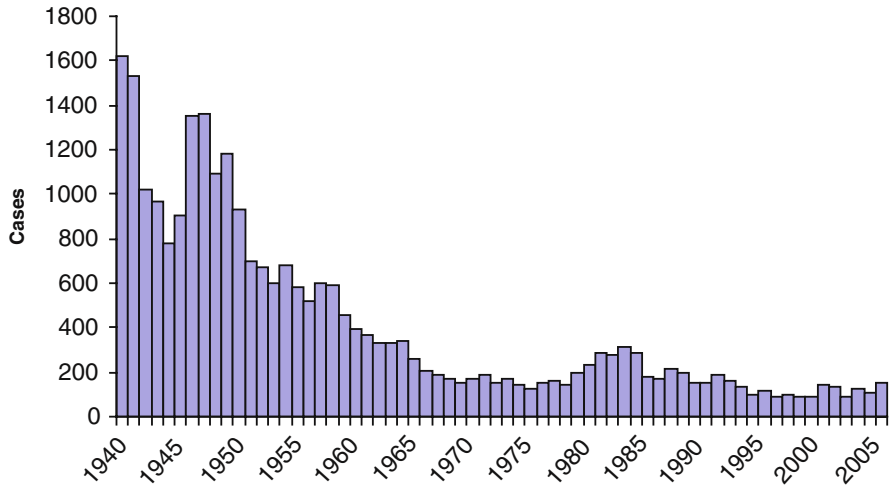


Figure 1. Reported tularemia cases, United States, 1940–2005.

the presence of an epizootic among rabbits, and *F. tularensis* was recovered from pooled collections of deer flies, tissues of jackrabbits and cottontail rabbits, and from rabbit ticks, *H. leporis-palustris*.

The first recognized tick-associated outbreak occurred in 1946 among soldiers on maneuvers in Tennessee.[53] Thirty-two of 50 infected soldiers reported a tick bite before illness onset. One patient died, and others had illness lasting as long as 6 months. Although the vector species could not be determined with certainty, all ticks collected in the area were *A. americanum*, an aggressive human-biting species. The dog tick, *D. variabilis,* has been implicated in outbreaks on Indian reservations in South Dakota[54,55] and Montana.[56] Interestingly, *D. variabilis* ticks collected from area dogs yielded both type A and type B *F. tularensis.*[54] In all three outbreaks, cases occurred primarily among Indian children and were associated with tick bites on the head or neck. Illness was relatively mild and characterized by cervical or occipital lymphadenopathy. The wood tick*, D. andersoni,* has been shown to transmit tularemia among rabbits,[57] but has not been linked to recognized outbreaks among humans. Mosquitoes have been implicated epidemiologically in summertime outbreaks in Scandinavia, but not in North America.[46] In a Swedish case–control study with 270 cases and 438 controls, mosquito bites were identified as the main risk factor, with an odds ratio of 8.8.[58]

Although animal contact usually occurs on an individual basis and produces sporadic infections, outbreaks due to animal exposure have been documented among specific populations. In the spring of 1968 in Addison County, Vermont, 47 cases of tularemia were diagnosed during a 4-week period among persons who had trapped or handled and skinned muskrats, including 87% of those trapping along a particular creek.[59] Thirty-nine of the cases were symptomatic and eight were asymptomatic seroconversions. No deaths were reported; however seven patients had chronic or reoccurring symptoms lasting several months. Wearing gloves while skinning the animals (or having someone else skin the animals) appeared to be protective. *F. tularensis* was isolated from muskrats, mud, and water collected from the stream. No ticks or fleas were recovered from 18 muskrats captured along the stream, supporting the notion that transmission among the muskrats occurred directly through ingestion of contaminated water or food. During the same year, muskrat-associated epizootics were reported in New York and Ontario.[59] Although less common, outbreaks have also been associated with trapping of aquatic invertebrates. In 1998, an outbreak of ulceroglandular tularemia caused by type B *F. tularensis* was reported in Spain among persons collecting freshwater crayfish.[60]

Waterborne transmission to humans was first described in the former Soviet Union, with outbreaks linked to both natural bodies of water and water from wells and municipal systems.[61] In 1936, Karpoff and Antonoff reported an outbreak among 43 field workers who developed tularemia, primarily oropharyngeal, after drinking from a stream while mowing hay.[9] Co-workers who drank from a neighboring stream remained well, and *F. tularensis* was later isolated from the implicated stream. A second outbreak occurred in a railway station and was linked to unchlorinated water piped from a stream, while another was attributed to water from a well which, on inspection, contained rodent carcasses. Unchlorinated water from municipal aqueducts has also been implicated as the source of outbreaks in Italy[62,63] and in

Turkey.[20] A seasonal association with respect to waterborne outbreak has been noted, with those occurring during the summer more often associated with natural bodies of water, whereas those occurring in the fall and winter months are more often associated with wells and piped water.[61]

Outbreaks due to aerosol exposure have occurred in several different settings. In 1959, hundreds of cases of pneumonic tularemia were documented among Austrian sugar factory workers.[28] The workers likely became ill through inhalation of aerosols generated during the mechanical washing and chopping of sugar beets, which that year were contaminated with the bodies of many dead field voles. In the winter of 1966, over 600 cases were reported in Sweden, primarily among farmers.[27] This epidemic was preceded by a large increase and sudden die-off in local vole populations. Hay stores of area farms were heavily contaminated with dead voles and their feces, and the dust generated by farmers while trying to clear these contaminants was considered the likely mode of transmission. Although some patients reported skin ulcers consistent with ulceroglandular tularemia, 32% reported sore throat, 26% conjunctivitis, and 11% symptoms of pneumonia. Overall, 83% reported contact with hay contaminated by voles. *F. tularensis* was recovered from voles, vole feces, and contaminated hay. Serologic studies revealed that over 20% of the population had been infected in some areas, with approximately a quarter of these infections being subclinical. In 1982 a similar outbreak of hay-associated pneumonic tularemia occurred among farmers in northern Finland.[64]

In North America, outbreaks of pneumonic tularemia have been described on Martha's Vineyard, an island off the southern coast of Massachusetts. Seven people developed pneumonic tularemia after staying together in a single cottage on the island in 1978.[65] Although the source of the outbreak could not be proven, the patients may have been infected when a pet dog, just back from catching rabbits in the rain, shook inside the cottage. More recently, an outbreak with 15 cases of pneumonic tularemia, including one fatality, was reported.[66] Type A *F. tularensis* was isolated from the fatal case. Patients were significantly more likely than controls to have mowed lawns or cut brush in the 2 weeks before their illness, but only one patient reported a known rabbit exposure. A serosurvey of island landscapers showed that 9% had evidence of past infection with *F. tularensis.* Elevated antibody titers were associated with mowing and leaf-blowing activities.[29] Smaller clusters of airborne transmission associated with mowing over sick or dead rabbits have been reported.[67]

Although *F. tularensis* can be transmitted through ingestion of contaminated foods, large outbreaks linked to commercial foodstuffs have not been reported. In the Czech Republic, 131 nursing home residents developed oropharyngeal tularemia after drinking juice made from fallen apples contaminated by rodents.[68] Smaller clusters include five cases attributed to eating strawberries,[69] and two cases of oropharyngeal tularemia in a couple who shared a meal of medium-cooked rabbit at a Berlin restaurant.[70]

5.3. Geographic Distribution

Tularemia occurs in regions throughout the northern hemisphere. In Europe and Asia, tularemia has been reported in Scandinavia, continental Europe, central Asia, Middle East, Russia, northern regions of China, and Japan, but not north of the arctic or in the British Isles.[42] In North America, *F. tularensis* has been found from northern Mexico to the arctic circle in Alaska.[42] This global distribution correlates with the holoarctic faunal zone and presumably relates to the distribution of vertebrate and arthropod species important for maintenance and transmission of the organism. In the United States, tularemia is reported most commonly in the south central states (Figure 2).

5.4. Seasonal Distribution

The seasonal distribution of human tularemia varies with the prevailing source of infection and can change over time with changes in human behavior. Studies from the United States typically reveal a bimodal distribution with winter and summer peaks due to infections acquired from rabbits and ticks, respectively.[68,71–73] Before 1950, the majority of cases occurred in winter. However, as rabbit hunting waned in the United States as a source of food and recreation, the total number of cases declined and the seasonality shifted toward that of a summertime disease.[74] Currently 70% of cases occur from May through August.[45] In Japan where exposure to hares remains the leading source of infection, the majority of cases continue to occur in the winter months.[48,75] In Scandinavia, most cases occur during the months of July through September with mosquitoes implicated in disease transmission.[27,76] Conversely, in a 40-year series from the Czech Republic, the majority of cases occurred from November through February, and contact with hares, rodents, or other mammals was reported as the likely source of infection.[68]

5.5. Age and Sex Distribution

The demographics of tularemia vary slightly from region to region reflecting differences in prevailing modes of transmission. Males account for the majority of cases in series from the United States,[45] Japan,[48] Finland,[76]

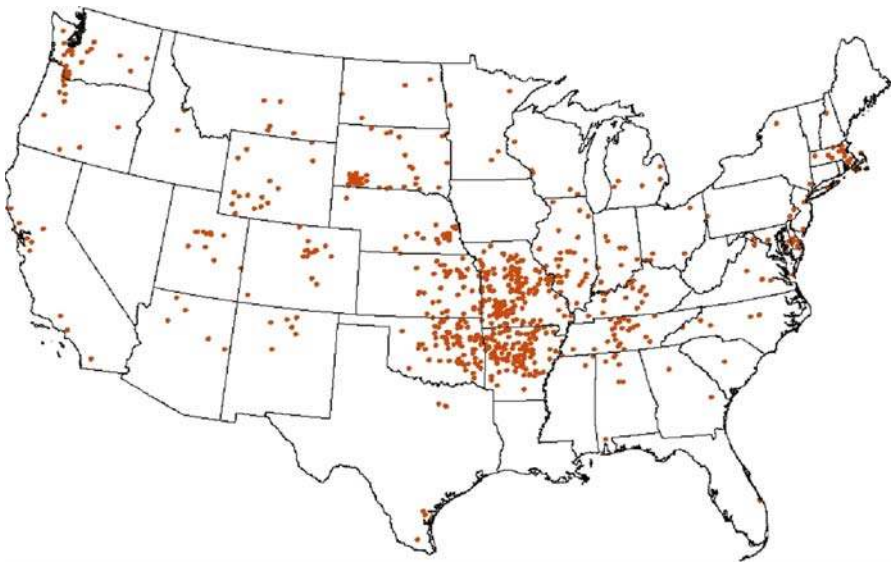


Figure 2. Reported cases of tularemia in humans, United States, 2000–2005.
1 dot placed randomly within country of residence for each reported case

and Sweden.[17] This higher incidence has been attributed to greater exposure through occupation or recreational activities (e.g., hunting).[58] Conversely, in a series from Turkey where waterborne cases of oropharyngeal tularemia predominate, 64% of identified cases were in females.[20] The age distribution of reported cases in the United States is bimodal, with highest rates among children aged 5–9 years and adults aged 55 and older.

5.6. Occupation and Recreation

As with many other zoonotic diseases, the risk of tularemia is closely linked to occupation and recreational activities. Hunters, trappers, sheep shearers, veterinarians, forest rangers, game wardens, hikers, campers, and others with frequent animal or arthropod exposure are at increased risk of infection. In a survey of Native Alaskan men, as many as 62% had positive skin tests for tularemia.[22] In some areas, farmers and landscapers appear to be at increased risk of pneumonic tularemia as a consequence of exposure to contaminated dust and aerosols.[27,64,27,66] *F. tularensis* is highly infectious when grown in culture, and numerous cases have been documented among laboratory workers.[77,78] Recently, a few cases have been linked to commercially traded pets, suggesting that persons in the pet industry may also be at increased risk.[79,80]

5.7. Other Factors

In the United States, the incidence of tularemia is approximately 10-fold higher among American Indians and Alaskan Natives as compared with whites. This difference is attributed to greater exposure to infected animals and arthropods among these ethnic groups.[45]

5.8. Occurrence in Different Settings

Although tularemia infections are generally more common in rural areas in association with outdoor activities that promote direct contact with infected animals or arthropods, cases occasionally occur in urban and other atypical settings. Five children living in urban Baltimore developed pneumonic tularemia after contact with their dog, which had caught and killed a rabbit in a nearby park.[81] Sporadic cases have also been reported among children living in inner cities.[82,83] Although rarely associated with human illness, several outbreaks in urban zoos, primarily among primates, have been reported.[84,85]

6. Transmission to Humans

Humans are highly susceptible to infection with *F. tularensis*. The average infectious dose for humans is estimated at 10 organisms by subcutaneous inoculation and 25 organisms by aerosol;[14,86] animal studies suggest that the infectious dose is orders of magnitude higher when the organism is ingested or applied to intact skin.[87–89] Person-to-person transmission of *F. tularensis* has not been demonstrated, and no special precautions are needed for managing infected patients.

Most human infections occur through cutaneous exposures. Biting flies act as mechanical vectors introducing the organism directly into the skin. During tick bite, *F. tularensis* may be inoculated directly with saliva or indirectly from tick feces. Hands may become contaminated when skinning or butchering an infected animal, tissues of which can contain up to 10^7/organisms per gram. The organism may then enter through small cuts in the skin or be transferred to the eye or other mucus membranes. Humans may also become infected through bites of infected mammals, especially cats,[90] or exposure to dogs that have caught a sick or dying rabbit.

Infection of the oropharynx is associated with ingestion of contaminated water or food, with few or no signs of gastrointestinal disease.[91] Inhalational exposures can occur during agricultural activities that may expose the individual to rodent contaminated dust. In Scandinavian outbreaks, manipulation of hay appears to be a common exposure,[64,92] and experimental studies have shown that *F. tularensis* can survive in hay for months when held at cold temperatures.[93] Aerosols may also be generated when animals are skinned or butchered. Perhaps the most extreme expression of this occurs when a sick or dead animal is accidentally or intentionally run over with a power mower, a practice that has given rise to the term "lawnmower tularemia."[67] In laboratory studies, the ability of aerosols to infect primates decreases rapidly over a few hours, even though viability of the organisms persists.[94] Whereas large particles may become trapped in the oropharynx,[95] particles 8 μm or smaller reliably cause pneumonia in nonhuman primates.[96,97]

7. Pathogenesis and Immunity

7.1. Pathogenesis

Within the vertebrate host, *F. tularensis* is considered an obligate intracellular pathogen[98] that replicates within macrophages, hepatocytes, and other cells.[99] Once introduced into the skin or mucus membranes, the bacteria multiply locally, producing an ulcerating lesion and spreading to the local lymph nodes. Infection causes follicular hyperplasia in lymph nodes which progresses rapidly to focal suppurative necrosis and ultimately granuloma formation. A bacteremic phase follows, resulting in seeding of other organs, especially liver, spleen, lung, and bone marrow. Following inhalational exposure a comparable process occurs, with destruction of bronchial tissues corresponding to skin ulceration and hilar enlargement corresponding to regional lymphadenitis.[92] Histologically, tularemia is characterized by granulomatous lesions with areas of focal caseous necrosis.[100] In autopsy series, caseating granulomas, grossly indistinguishable from those caused by tuberculosis, have been found in the lungs, spleen, lymph nodes, and bone marrow.[99]

Virulence factors for *F. tularensis* are largely unknown. The lipopolysaccharide (LPS) lacks classic biologic activity of endotoxin in stimulating cytokine production.[101] A capsule encases the bacterium and helps protect against complement-mediated killing in the serum; however, it is not necessary for survival following phagocytosis.[102] *F. tularensis* enters macrophages without triggering a respiratory burst, an ability that appears related to the activity of AcpA, a protein with acid phosphatase activity. Within the macrophage, *F. tularensis* multiplies within the phagosome. The phagosome does not fuse with lysosomes but does acidify, thus producing an environment essential for the growth and acquisition of iron by the organism. No secreted toxins have been identified.[101]

7.2. Immunity

LPS in the capsule and other proteins of *F. tularensis* elicit a humoral response that can be used diagnostically to demonstrate infection. Antibody titers peak approximately 6 weeks after illness onset and may remain elevated for several years. As expected for an intracellular pathogen, antibody appears to play only a minor role in resistance to infection relative to cell-mediated immunity.[99] In animal models, passive transfer of mononuclear leukocytes or T lymphocytes from immunized animals protected against challenge with virulent strains, whereas immune serum did not. In animal models, protection against virulent strains requires CD4+ and CD8+ T-cell responses, although recent work suggests that other populations may also have a role.[103] Studies using B-cell knockout mice confirm that animals are only marginally compromised in controlling primary sublethal infection and suggest another role for B cells in secondary immunity independent of antibody production.[104] Once induced, cell-mediated immune responses persist for long periods. In a follow-up study of naturally infected patients, T-cell response to *F. tularensis* remained vigorous 25 years later.[105] Although re-infection has been documented, it is extremely rare.[99]

8. Patterns of Host Response

In humans the clinical manifestations of *F. tularensis* infection range from subclinical to life-threatening. The clinical presentation is determined by the route of exposure, the dose, and the infecting strain. The average incubation

period is 3–6 days, but ranges from 1 to more than 14 days. For those who become ill, clinical course and outcome are strongly influenced by host characteristics and the timeliness and appropriateness of medical care.[106]

8.1. Clinical Features

Six primary clinical syndromes have been described: ulceroglandular, glandular, oculoglandular, orophoryngeal, pneumonic, and typhoidal tularemia. All forms are characterized by sudden onset of fever, chills, headache, malaise, fatigue, myalgias, and arthralgias. Fever can be high[104] with a paradoxically slow pulse. Skin manifestations have been reported in 8–40% of patients and include vesiculopapular eruptions, erythema nodosum, and erythema multiforme.[107–113] White blood cells counts may or may not be elevated.[112,114]

Ulceroglandular tularemia is the most commonly reported clinical syndrome in most areas and results from cutaneous exposure through arthropod bites or direct contact with tissues or secretions of an infected animal.[107,115,107,116] A small papule appears concurrent with the onset of fever. Over the next few days, regional adenopathy develops and the skin lesion evolves into an ulcer (Figure 3). Usually solitary, the ulcer may develop satellite papules and pustules. The regional lymph nodes become tender and enlarged with erythema of the overlying skin. Suppuration was common prior to the availability of antimicrobial treatment. A small proportion of patients develop secondary pneumonia, although this is generally mild.[112] During the latter stages of infection, the spleen may be enlarged. The differential diagnosis for ulceroglandular tularemia includes bubonic plague, sporotrichosis, *Mycobacterium marinum* infection, chancroid, tick typhus, anthrax, and staphylococcal lymphadenitis. Glandular tularemia is similar to ulceroglandular disease, but without an obvious cutaneous ulcer. In addition to the diseases mentioned above, glandular tularemia may be confused with cat-scratch disease, tuberculosis, toxoplasmosis, and lymphogranuloma venereum.



Figure 3. Ulcer on thumb of a patient with ulceroglandular tularemia.

Oculoglandular tularemia is believed to result from direct inoculation of organism into the eye, either from contaminated fingers or possibly through aerosols.[4,72,4,117,118] Historically, this has been associated with skinning and butchering rabbits.[4,119] Currently, oculoglandular tularemia accounts for fewer than 5% of reported cases.[107,115,107,116] The patient usually presents with fever, unilateral conjunctivitis with purulent discharge, swelling of the eyelids, and necrotic yellow ulcers or pustules on the palpebral conjunctivae. Adjacent lymph nodes, including preauricular, submandibular, and cervical, may be tender and enlarged. In the original case report by Vail, a pustular eruption was noted on the skin adjacent to the eye.[4] Corneal edema and glaucoma have been reported as complications.[120]

Oropharyngeal tularemia is associated with ingestion of contaminated water or food.[69] Occasionally, it may occur after an inhalational exposure. Patients complain of sore throat and neck swelling. Physical examination reveals pharyngitis which may be exudative or accompanied by small ulcers. There is marked cervical adenopathy, but it may be unilateral. Without effective treatment, suppuration is common.[20] Differential diagnoses include viral and streptococcal pharyngitis, diphtheria, mumps, and the conditions mentioned above that may mimic cervical glandular tularemia.

Pneumonic tularemia can be either primary, following inhalation of the organism, or secondary, following hematogenous spread to the lungs from other areas of the body.[121] Many reported series include both,[122,123] obscureing potential differences in their clinical features. Regardless, the presentation is not readily distinguished from other forms of community-acquired pneumonia, underscoring the importance of a careful exposure to history. Symptoms include fever, headache, dry paroxysmal cough, pleuritic or retrosternal pain, and dyspnea. A wide variety of radiographic findings have been described, including lobar and multilobar infiltrates, lung abscess, and hilar adenopathy.[112,115,112,122,124] Pleural effusions have been reported in 20–30% of cases, and by most reports appear to be transudative.[112,122,112,123] The differential diagnosis includes community-acquired pneumonias caused by *Mycoplasma*, *Chlamydia*, *Legionella*, *Histoplasma*, and common bacterial and viral agents. Additional etiologies to consider are Q-fever, inhalational anthrax, pneumonic plague, and tuberculosis. Because pneumonic tularemia can be

severe, it should be considered in the differential diagnosis of any patient with community-acquired pneumonia.

The term typhoidal tularemia is found throughout the literature and was originally used to designate patients with systemic infection but no skin lesions, lymphadenopathy, or other localizing signs. At the time, transmission through ingestion and inhalation was not recognized. Review of historical series reveals that many patients diagnosed with typhoidal tularemia had or developed respiratory symptoms,[107,125] and it is possible that these patients would today be classified as having primary pneumonic tularemia.[121] Similarly, some series describe typhoidal tularemia patients with oropharyngeal involvement.[115] Given current understanding about the routes of infection, the term typhoidal tularemia may be outmoded.[92] Clinically, typhoidal tularemia may be confused with any other cause of fever without localizing signs including typhoid fever, brucellosis, Q-fever and other rickettsial infections, infectious mononucleosis, toxoplasmosis, cytomegalovirus disease, babesiosis, malaria, and bacterial endocarditis.

Rare manifestations of tularemia include meningitis,[126,127] pericarditis,[128] endocarditis,[129] and infections of prosthetic devices.[130,131] Although experience with tularemia in immunocompromised patients is limited, patients with impaired cell-mediated infection may be especially susceptible. Sporadic cases of *F. tularensis* infection without an apparent source of infection have been reported in patients with HIV infection,[132] neutropenia following bone marrow transplant,[133] chronic granulomatous disease,[134] stomach cancer,[135] and in patients receiving immunosuppressive therapy.[136,137]

8.2. Laboratory Diagnosis

Laboratory staff must be notified if tularemia is suspected, both to assure optimal testing and to reduce the risk of laboratory-acquired infection. Laboratory diagnosis is usually based on isolation of the organism by culture or detection of elevated antibody titers. Additional testing methodologies are available through public health and reference laboratories, including antigen detection and PCR. Isolates may be subtyped using a variety of molecular techniques (MLVA, PFGE, SNPs), with potential applications in epidemiologic and ecological studies. Gram staining of clinical specimens can be useful to help exclude other etiologies; however the small size of *F. tularensis* makes it difficult to identify by light microscopy.

8.2.1. Isolation and Identification of the Organism. Most clinical isolates are fastidious and require cysteine or cystine for optimal growth. Specimens from enriched sources such as tissue or blood can be plated on sheep blood agar, but recovery from other specimens is best accomplished using chocolate agar, Thayer–Martin agar, cystine heart agar with 9% chocolatized blood (CHAB), buffered charcoal yeast extract, or other cysteine-supplemented agar. Specimens should be incubated at 35–37°C and held for 5 days. On CHAB, *F. tularensis* produces characteristic green or opalescent shiny colonies, allowing for presumptive identification after 24–48 h. Recovery from contaminated or mixed flora specimens can be enhanced using CHAB media supplemented with antibiotics (CHAB-A).[138]

Depending on the clinical manifestations, specimens for culture may include ulcer scrapings or swabs, tissue (e.g., lymph node), lymph node aspirates, sputum, pleural or bronchioalveolar lavage fluid, pharyngeal washes, or gastric aspirates. Recovery of *F. tularensis* from blood culture is uncommon but may be enhanced by use of the BACTEC or equivalent system.[139,140] Primary specimens should be evaluated using Biological Safety Level 2 (BSL-2) precautions; BSL-3 precautions are recommended for work with all suspect cultures. In the United States, the state public health laboratory should be notified if any isolate is suspected of being *F. tularensis*.

Confirmation is based on recovery of an isolate and identification of the culture as *F. tularensis* by antigen detection or PCR.[141] Antigen detection methods include direct fluorescent antibody staining (DFA), slide agglutination, and ELISA.[142] DFA can also be used directly on tissues to yield a presumptive diagnosis of tularemia. Conventional PCR and multitarget real-time TaqMan PCR assays have been developed and are available at some reference laboratories.[141] Automated biochemical identification systems are not recommended for culture identification as they may misidentify the organism.

Once an isolate has been confirmed as *F. tularensis*, supplemental tests can be performed in reference laboratories to identify subspecies and antimicrobial susceptibilities. In Europe, clinical strains can be safely assumed to be type B; however, in North America, both type A and type B commonly cause human infection. *F. tularensis* ssp. *tularensis* (type A) can be differentiated from *F. tularensis* ssp. *holarctica* (type B) strains based on the ability to ferment glycerol and convert L-citrulline to ornithine. Subspecies can also be differentiated using conventional and real-time PCR.[143,144] For susceptibility testing, broth microdilution using cation-adjusted Mueller–Hinton broth (CAMHB) with 2% defined growth supplement can be used (Clinical and Laboratory Standards Institute, 2005). Naturally occurring resistance to antibiotics commonly used for treatment has not been reported.

8.2.2. Serological and Immunological Tests. Because culture of *F. tularensis* can prove difficult, serology is often the basis for laboratory confirmation. Combined immunoglobulin M (IgM) and immunoglobulin G (IgG) against *F. tularensis* can be detected by microagglutination or tube agglutination using whole killed cells.[145,146] A rise in antibody titer is usually detectable within 2–3 weeks of illness onset and may remain for 6–8 months before declining.[147] A single titer of $\geq$1:160 by tube agglutination or $\geq$1:128 by microagglutination is indicative of infection; however, confirmation of recent infection requires a fourfold change in titer between paired specimens, with at least one specimen having a positive titer. Cross-reactions with antibody to *Brucella* have been described.[148] ELISAs directed at LPS are also available, and ELISA combined with Western blot has been shown to have excellent sensitivity and specificity.[149]

8.3. Prognosis

Before the advent of effective antimicrobial therapy, tularemia was characterized by a prolonged convalescence lasting 3 months or longer.[119] When treated promptly with streptomycin, patients improve quickly, some becoming afebrile within 24 h, and most recover completely.[112] Treatment with bacteriostatic agents, including chloramphenicol and tetracyclines, has been associated with relapse if treatment periods are not long enough to allow development of a cell-mediated immune response.[150,151] Other factors associated with poor outcome include underlying medical conditions and delay in seeking treatment.[106] In a recent series where treatment was delayed an average of 8 weeks, 22% of patients treated with streptomycin and 43% treated with doxycycline had persistent or relapsing symptoms.[116] Reported complications of tularemia include suppuration of infected lymph nodes requiring drainage, hepatic abscess,[152] hepatitis,[112,153] renal failure,[106,154] rhabdomyolysis,[139] and adult respiratory distress syndrome.[155]

Estimates of mortality are complicated by differences in the virulence of the two *F. tularensis* subspecies. In Eurasia where only type B strain cause infection, fatal cases are rare. Soviet scientists established early a low mortality rate (<1%) in the USSR due to type B infections.[10] Similarly, no deaths were reported among 1,355 cases reported in Japan from 1924 to 1987,[18] or among 205 cases reported in Turkey from 1988 to 1998, despite the fact that approximately one third were not treated with antimicrobials.[20]

In North America the situation is more complicated as both type A and type B cause illness yet subspecies identification is rarely undertaken. Mortality for some clinical forms can be quite high in the absence of treatment. Five of 123 patients cared for by Kavanaugh before 1932 died, yielding a case fatality rate of 4% overall and 25% among patients with pulmonary involvement.[118] Seventeen (7%) of 225 patients diagnosed at New Orleans Charity hospital before 1946 died, including 12 (57%) of 21 patients with pneumonic involvement.[107] In these and most other early series, pneumonic involvement includes patients with primary pulmonary tularemia and those who developed pneumonia as a complication of other forms of infection, usually ulceroglandular tularemia.

With the advent of antimicrobial treatment, specifically streptomycin, tularemia mortality decreased substantially in the United States. In a review of 339 cases from Louisiana, mortality among patients with pneumonic involvement was only 18% in streptomycin-treated patients, as compared with 80% before the advent of streptomycin.[115] No deaths occurred among 28 Arkansas patients with pneumonic tularemia treated with streptomycin between 1949 and 1969.[122] In a series from the 1980s, four (12%) of 32 patients with pneumonic involvement died, although it is not clear what type of therapy each received. Overall, expected mortality for appropriately treated patients is generally estimated at <1% overall and <5% for patients with pneumonic tularemia.[13]

To better define mortality rates associated with type A and type B infections in the United States, a retrospective study compared outcome for 308 cases for which isolates were submitted to CDC between 1964 and 2004.[38] Contrary to expectations, overall mortality rates were similar: 7% for type B and 9% for type A. Molecular subtyping using pulsed-field gel electrophoresis revealed that type A strains clustered into two distinct genetic groups, one found primarily in the eastern United States and the other primarily in the western United States. Mortality associated with type A east infections was 14%, as compared with 7% for type B infections and 0% for type A west infections. Although the absolute magnitude of mortality in this study is likely elevated due to referral bias, the data suggest a relative difference between the three groups. It remains to be determined whether these differences are related to differences in the virulence of the organism or other factors, such as route of exposure.

9. Control and Prevention

9.1. General Concepts

Prevention of tularemia takes many forms, depending on the likely route of exposure. Use of protective clothing, repellents, or pesticides may help reduce risk of exposure from ticks or other arthropods. Hunters or others who handle

potentially infected animals or tissues should wear gloves to avoid getting the bacteria through cuts or abrasions on their hands. Game meat should be cooked thoroughly and care taken to avoid cross-contamination of other items in the kitchen. Rodents should be excluded from food and water supplies, and drinking water should be chlorinated to reduce risk of infection.

There is scant information on the efficacy of masks or respirators for preventing pneumonic tularemia, especially under field conditions. Use of high-efficiency masks may be prudent when handling hay or other material contaminated by rodents, especially if there is evidence of a die-off in the area. The risk of lawnmower tularemia may be reduced by surveying areas beforehand for animal carcasses and avoiding any dead animals encountered when mowing.

To prevent laboratory-acquired infections, laboratory staff should be notified whenever tularemia is suspected. Processing of clinical samples should be conducted using Biological Safety Level 2 (BSL-2) procedures. BSL-3 procedures should be employed when manipulating cultures or for any activity that could potentially create an aerosol. Isolates presumptively identified as *F. tularensis* at a BSL-2 laboratory should at a minimum be handled in a biosafety cabinet and preferably transferred to a BSL-3 laboratory for further evaluation. In the event of a laboratory exposure, the options are immediate antimicrobial prophylaxis with either ciprofloxacin or doxycycline for 14 days,[13] or a "fever watch" with prompt treatment upon development of fever or other signs of infection within 2 weeks of exposure. Factors to consider in choosing between these options include the number of days elapsed since exposure, the likelihood of an aerosol exposure, and worker anxiety.

Person-to-person transmission of tularemia has never been demonstrated. Therefore, standard hospital infection precautions are considered adequate when managing patients with tularemia.[13]

9.2. Chemotherapeutic Approaches to Prophylaxis

F. tularensis isolates are susceptible to aminoglycosides, tetracyclines, and fluoroquinolones but resistant to many beta-lactams including penicillin, cefuroxime, and imipenem.[13] For treatment of tularemia, streptomycin as long been considered the drug of choice. Treatment with another aminoglycoside, gentamicin, has been more common in recent years. The dose of streptomycin for an adult is 1 g intramuscularly twice daily, and for a child 15 mg/kg twice daily (up to a maximum daily dose of 2 g).[13] Gentamicin may be given intravenously in a dose of 5 mg/kg/day for adults and 7.5 mg/kg/day in children. Several case reports suggest that ciprofloxacin is also effective, although it is not FDA approved for this indication.[13] Streptomycin, gentamicin, and ciprofloxacin should generally be given for 10 days. Bacteriostatic agents, such as tetracyclines, have also been used but are associated with a higher risk of relapse and require longer courses of treatment. Oral or intravenous doxycycline can be effective if given for 14–21 days in a dose of 100 mg/kg twice a day for adults and 2.2 mg/kg twice a day up to a maximum daily dose of 200 mg for children. Ceftriaxone should not be used as several treatment failures have been reported.[151] For prophylaxis following a high-risk exposure, doxycycline or ciprofloxacin is recommended.[13] No natural resistance to antibiotics used for treatment has ever been documented.

9.3. Immunization

The best-studied vaccine for tularemia is the attenuated live vaccine strain (LVS). Developed in the United States using an attenuated type B strain from the Soviet Union, LVS is not licensed for general use. LVS has been evaluated in studies conducted by the United States Department of Defense and is immunogenic when administered intradermally, orally, or by aerosol.[91,156] In aerosol challenge studies using up to 20,000 type A organisms, volunteers immunized intradermally with LVS were protected from infection or had milder illness than controls.[157] Retrospective analysis of laboratory-acquired infections among Department of Defense employees revealed that the incidence of typhoidal tularemia fell from 5.70 to 0.27 cases per 1,000 at-risk employee-years following vaccination with LVS. The incidence of ulceroglandular tularemia remained unchanged; however clinical signs and symptoms were milder.[158] Despite this evidence of efficacy, the protective components of LVS have not been defined, and due to difficulties with standardization, LVS has not been licensed for general use. Development of alternative vaccines is an area of active research.

10. Unresolved Issues

Although virulence differences among strains of *F. tularensis* are well documented, the genetic and molecular determinants of these differences are largely unknown. The ecologic factors underlying the sympatric distribution of different strains of *F. tularensis* are similarly unknown, as are the events that trigger epizootics among host species. Finally, it remains a considerable paradox as to why human-to-human transmission does not occur with such an otherwise highly infectious, airborne pathogen.[159]

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CHAPTER 43

Typhoid Fever

Myron M. Levine

1. Introduction

Typhoid fever is an acute generalized infection of the reticuloendothelial system, intestinal lymphoid tissue, and gallbladder caused by *Salmonella enterica* serovar Typhi (*S.* Typhi). This communicable disease is restricted to human hosts and humans (chronic carriers) serve as the reservoir of infection. A broad spectrum of clinical illness can ensue, with more severe forms being characterized by persisting high fever, abdominal discomfort, malaise, and headache. In the preantibiotic era, the disease ran its course over several weeks, resulting in a case fatality rate of approximately 10–20%.[1,2] The protracted, debilitating nature of this febrile illness in untreated (or improperly treated) patients is accompanied by mental cloudiness or stupor, which gave rise to the term "typhoid," meaning stuporlike. Paratyphoid fever is the clinically similar febrile infection caused by *S.* Paratyphi A or B (or more rarely C). Typhoid and paratyphoid fevers are also referred to as enteric fevers. In most endemic areas, typhoid comprises approximately 80% of enteric fever.

Typhoid infection is virtually always acquired by ingestion of food or water vehicles contaminated by human excreta that contains *S.* Typhi. Typhoid fever is extremely uncommon in modern industrialized countries where populations have access to treated water supplies and sanitation that removes human waste. In contrast, among populations in less-developed countries that lack such amenities, typhoid fever is often endemic and, from the public health perspective, typically constitutes the most important enteric disease problem of school-age children.[3] Besides school-age children in less-developed countries, two other populations are at recognized risk of typhoid: travelers and clinical microbiologists. Typhoid fever occurs with variable frequency among travelers from industrialized countries who visit less-developed areas of the world. Among US travelers, the risk is highest with travel to the Indian subcontinent, Mexico, Central America, the Philippines, and Haiti.[4,5] Clinical microbiologists, including those in industrialized countries, have increased exposure to *S.* Typhi and therefore also constitute a high-risk group.[6,7]

Typhoid fever is readily treated by prompt administration of appropriate antibiotics if strains are susceptible.[8] Since 1990, strains of *S.* Typhi exhibiting resistance to most of the antimicrobials that previously were clinically effective have spread aggressively throughout the Indian subcontinent, Southeast Asia, and the Middle East.[9–12] A few antibiotics that are effective against these multiply resistant strains are expensive and not readily available in rural areas of less-developed countries. Thus, the arrival on the scene and dissemination of these multiply resistant *S.* Typhi constitute an increasing public health crisis in many less-developed countries.

2. Historical Background

Prior to the early decades of the nineteenth century, typhoid fever was not recognized as a distinct clinical entity and often was confused with other prolonged febrile syndromes, particularly typhus fever of rickettsial origin. Debate continues over who first clearly differentiated typhus fever from typhoid (i.e., typhus-like) fever,[13] as medical historians have variously bestowed credit for this clinical clarification upon Huxham (1782),[13a] Louis (1829),[13b] Gerhard (1837),[13c] and Schoenlein (1839).[13b,d]

In 1829, Bretonneau[13e] described a severe enteric infection occurring in young Parisians and used the term "do-thientérite" ("boil of the intestine") to describe

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the lesion that can lead to intestinal perforation. Louis catalogued the abnormal lesions in the intestine, lymph nodes, spleen, and skin (rose sports) in 158 patients and describes hemorrhage and perforation in the gut. During this period, the intestinal lesions were thought to be a complication or unusual extension of typhus fever.

Gerhard,[13c] working in Philadelphia, contended that two similar yet distinct febrile illnesses exist that are nevertheless discernable from one another on the basis of pathological findings: one (typhoid) exhibits prominent intestinal lesions. Schoenlein referred to two distinct forms of typhus, "exanthematicus" and "abdominalis." However, it is William Jenner who, circa 1850, definitively dispelled controversy on the subject by providing precise clinical descriptions and pathological observations from postmortem examinations that allowed a clear-cut differentiation between the two illnesses. Jenner argued that the pathological lesions in Peyer's patches and mesenteric lymph nodes were peculiar to typhoid and were never seen with typhus. In 1847, the term "enteric fever" was introduced in an attempt to replace typhoid fever and avoid confusion with typhus. While this term is frequently used, it never supplanted the more common usage of typhoid and paratyphoid fever.

In William Budd's 1873 milestone in epidemiology,[14] *Typhoid Fever: Its Nature, Mode of Spreading and Prevention*, he clearly described the contagious nature of the disease and incriminated fecally contaminated water sources in transmission, years before the causative organism was identified. Eberth (1880)[13f] visualized the causative bacilli in tissue sections from Peyer's patches and spleens of infected patients, while Gaffky (1884)[13g] grew it in pure culture. Known in earlier years as *Bacillus typhosus, Eberthella typhosa*, and *S. typhosa*, it is presently referred to as *S.* Typhi; the lack of italics and capital T indicates that taxonomically this bacterium is now considered a serovar of *S. enterica* and not a separate species.[15]

Typhoid fever was endemic in virtually all countries of Europe and North and South America in the late nineteenth and early twentieth centuries. A single intervention – the treatment of water supplies (by chlorination, sand filtration, or other means) – which became increasingly popular in the late nineteenth and early twentieth centuries, broke the cycle of endemicity and caused the incidence of typhoid fever to plummet drastically, even though the prevalence of chronic carriers in the populations remained high for decades thereafter.[16,17]

Inactivated (heat-killed, phenol-preserved) *S.* Typhi were utilized as parenteral vaccines as far back as 1896 by Pfeiffer and Kolle in Germany and Wright in England.[13] Wright administered his vaccine (three doses 2 weeks apart) to two medical officers in the Indian Army, one of whom thereafter ingested wild typhoid bacilli without developing illness. Thereafter, Wright tested his vaccine in 2,835 volunteers in the Indian Army. While local and generalized adverse reactions were common, the results were considered to be sufficiently encouraging for a decision to be made to vaccinate troops embarking for the Boer War in South Africa. Outcry over the frequency of adverse reactions led to a suspension of vaccination. However, on Wright's insistence, a board of enquiry was assembled to review data on the reactogenicity and efficacy of the vaccine. The committee concluded that the vaccine was efficacious and that its value in preventing typhoid fever exceeded the price paid in adverse reactions. Consequently, by World War I, typhoid vaccination became virtually routine in the British Army.

Randomized, placebo-controlled, field trials in the 1960s established the efficacy of the heat-phenolized and acetone-inactivated parenteral whole cell vaccines.[18–22] Although these vaccines were shown to confer moderate protection that persisted for up to 7 years, they never became well-accepted public health tools because they frequently caused systemic adverse reactions (about 25% of recipients developed fever and malaise).[18,19,23] The parenteral killed whole cell vaccines have been supplanted by two newer vaccines: attenuated *S.* Typhi strain Ty21a used as a live oral vaccine and purified Vi capsular polysaccharide utilized as a parenteral vaccine.[3] These two newer vaccines, which are at least as effective as the parenteral whole cell vaccines but are well tolerated, are licensed and available in many countries and constitute credible public health tools.

3. Methodology

In the United States, the occasional sporadic cases and rare outbreaks of typhoid fever that occur are regarded as infectious disease calamities that rivet the attention of public health officials. Extensive investigative efforts are mobilized to identify the individual, usually an asymptomatic carrier,[24,25] who has served as a source of the outbreak or of sporadic cases (when they occur among persons who are not recently returned travelers). Carriers are carefully identified and regularly followed by city or county health departments. Such monitoring ensures that carriers do not participate in occupations wherein they might contaminate food or water consumed by the public at large.

In less-developed countries where typhoid fever is endemic and public health resources are scarce, epidemiological investigations may be initiated only when a large-scale outbreak occurs or there is an unusual increase in the number of sporadic endemic cases.[26]

3.1. Sources of Mortality Data

Information on deaths from typhoid fever in the United States derives from two sources, the first of which is notifications published within the *Morbidity and Mortality Weekly Report* (MMWR). The second source, available since 1975, is the Centers for Disease Control and Prevention (CDC) typhoid case report form, which is completed by state and community health departments on all bacteriologically confirmed cases of typhoid fever.

Before antibiotic therapy was available, a proportion of typhoid deaths were attributed to intestinal hemorrhage or perforation followed by peritonitis. Other patients in that era died of complications rarely seen today, such as myocarditis, pneumonia, and meningitis.[1] However, most fatal cases of typhoid fever resulted from inanition consequent to several weeks of unremitting fever and obtundation without a recognized specific complication.

In the preantibiotic era, a more accurate estimate of the relative importance of typhoid fever as a public health problem could be obtained by consideration of typhoid mortality rates per 10^5 population than by reviewing incidence rates. This was true because milder cases of typhoid fever can be confused with other febrile syndromes and bacteriological confirmation was undertaken in only a small proportion of suspect cases; in contrast, typhoid deaths are fairly definitive. Indeed, if autopsy is performed, the ileal lesions of gut-associated lymphoid tissue are pathognomonic of typhoid or paratyphoid. Beginning in 1913, epidemiological data on typhoid mortality in large cities in the United States were summarized annually in the *Journal of the American Medical Association*.[17] In the period 1911–1915, annual typhoid death rates in the nine most populous US cities (New York, Chicago, Philadelphia, Detroit, St. Louis, Boston, Pittsburgh, Cleveland, and Baltimore) ranged from $8.0/10^5$ (New York, Boston) to $23.7/10^5$ (Baltimore).[17] Over the next decade it was possible to monitor the extraordinary effect that the rapidly increasing availability of treated water supplies had on diminishing typhoid mortality.[16]

The modern antibiotic era of typhoid epidemiology was ushered in by Woodward et al.[27] in 1948, when they demonstrated that chloramphenicol was highly effective in the treatment of this disease. Over the next 25 years, widespread use of this oral, inexpensive, effective antibiotic markedly diminished the case fatality rate of typhoid fever. Prior to the advent of chloramphenicol therapy, the case fatality rate of typhoid fever typically ranged from 10 to 20%, irrespective of where cases occurred worldwide. Presently, deaths from typhoid in the United States are distinctly rare, with only two deaths reported in the period 1996–2001 (one death in 1996 and the other in 2001).[28] Ironically, since antibiotics have become widely available worldwide, mortality statistics are no longer a reliable measure of the endemicity of typhoid fever in populations.

3.2. Sources of Morbidity Data

The vast majority of cases of typhoid fever diagnosed by clinicians in the United States are reported to state or local health departments. Many cases are reported directly by physicians. In addition, all positive stool, blood, bile, bone marrow, or other cultures that yield *S.* Typhi are reported by the laboratories making the isolations. The same two reporting systems mentioned above with respect to mortality also provide morbidity data.

In many less-developed countries with endemic disease, incidence data on typhoid tend to be unreliable and are distortions of the true disease burden; these distortions are usually under estimations but may also be over estimations. Many "fever" hospitals in developing and transitional countries record the number of patients admitted with the clinical diagnosis of typhoid fever, while others that have bacteriological capability report just the number of confirmed cases. The true incidence of typhoid fever in many less-developed countries is not known because the paucity of bacteriological capability precludes most suspected cases from being confirmed; moreover, suppression of disease by self-medication with antibiotics or by health practitioner treatment without obtaining cultures and incomplete reporting further distort the true situation.

3.3. Surveys

Several types of surveys have been carried out in recent years to study the epidemiology of endemic or epidemic typhoid. These include the following:

1. Bacteriological surveys of exposed populations during outbreaks[24,26,29] or household contacts of sporadic cases,[30] in order to detect subclinical and mild cases and carriers.
2. Systematic collection of blood cultures among febrile patients to confirm the incidence of *S.* Typhi infection in certain populations (Table 1).[31–37]
3. A combination of serological screening tests (for elevated serum titers of Vi antibodies) and bacteriological confirmation (by means of multiple stool cultures and bile-stained duodenal fluid cultures) to detect chronic biliary carriers of *S.* Typhi.[24,38–42]
4. Serosurveys to measure the prevalence of H antibodies in different age groups, as a measure of the cumulative contact with *S.* Typhi.[43,44]

Surveys of family members and persons known to be associated with an outbreak of typhoid fever are usually performed by culturing three successive stool specimens from asymptomatic subjects. One or more of these three

Table 1. The Mean Annual Incidence of Typhoid Fever Recorded in the Control Groups in Representative Vaccine Field Trials or in Population-Based Surveillance in South America, Asia and Africa

Country	Vaccine tested	Year	Surveillance method	Age group	Annual rate/10^5	References
Area Norte, Santiago, Chile	Ty21a	1982	Passive	6–19 years	227	(49)
Area Occidente, Santiago, Chile	Ty21a	1983–1986	Passive	6–9 years	116	(47)
Area Occidente, Santiago, Chile	Ty21a	1983–1986	Passive	10–14 years	110	(47)
Area Occidente, Santiago, Chile	Ty21a	1983–1986	Passive	15–19 years	87	(47)
Plaju, Indonesia	Ty21a	1986–1989	Passive	3–6 years	1,307	(52)
Plaju, Indonesia	Ty21a	1986–1989	Passive	7–19 years	1,172	(52)
Plaju, Indonesia	Ty21a	1986–1989	Passive	20–44 years	182	(52)
Mekong Delta, Vietnam	None	1995–1996	Passive	<2 years	0	(35)
Mekong Delta, Vietnam	None	1995–1996	Passive	2–4 years	358	(35)
Mekong Delta, Vietnam	None	1995–1996	Passive	5–9 years	531	(35)
Mekong Delta, Vietnam	None	1995–1996	Passive	10–14 years	429	(35)
Mekong Delta, Vietnam	None	1995–1996	Passive	15–19 years	153	(35)
Mekong Delta, Vietnam	None	1995–1996	Passive	20–29 years	149	(35)
Mekong Delta, Vietnam	None	1995–1996	Passive	30–39 years	51	(35)
Mekong Delta, Vietnam	None	1995–1996	Passive	34–49 years	39	(35)
Mekong Delta, Vietnam	None	1995–1996	Passive	≥50 years	27	(35)
Guangxi, China	Vi	1995–1996	Passive	5–9 years	14	(53)
Guangxi, China	Vi	1995–1996	Passive	10–14 years	25	(53)
Guangxi, China	Vi	1995–1996	Passive	15–19 years	32	(53)
Guangxi, China	Vi	1995–1996	Passive	20–25 years	13	(53)
Kolkata, India	None	2004	Modified passive	<1 year	0	(37)
Kolkata, India	None	2004	Modified passive	1 year	100	(37)
Kolkata, India	None	2004	Modified passive[a]	2 years	200	(37)
Kolkata, India	None	2004	Modified passive[a]	3 years	170	(37)
Kolkata, India	None	2004	Modified passive[a]	4 years	320	(37)
Kolkata, India	None	2004	Modified passive[a]	5–9 years	300	(37)
Kolkata, India	None	2004	Modified passive[a]	10–19 years	530	(37)
Kolkata, India	None	2004	Modified passive[a]	20–29 years	300	(37)
Kolkata, India	None	2004	Modified passive[a]	30–39 years	80	(37)
Nepal	Vi	1986–1987	Active	5–44	654	(51)
South Africa	Vi	1985–1987	Active	5–15 years	472	(50)
Mekong Delta, Vietnam	Vi conjugate	1998–2000	Active	2–5 years	414	(54)
Kalkaji, New Delhi, India	None	Nov 1, 1995–Oct 31, 1996	Active[b]	0–4 years	2,730	(32)
Kalkaji, New Delhi, India	None	Nov 1, 1995–Oct 31, 1996	Active[b]	5–19 years	1,170	(32)
Kalkaji, New Delhi, India	None	Nov 1, 1995–Oct 31, 1996	Active[b]	20–39 years	110	(32)

[a]The investigators established multiple (no-cost) treatment centers in the urban area under surveillance to facilitate febrile patients seeking health care and blood cultures were collected at these centers. They also arranged, as necessary, transport of patients to hospital.
[b]Residents of the Kalkaji cohort were visited twice weekly in their households to detect persons with fever. For persons <5 years of age a blood cultures was obtained if the temperature was ≥38°C, irrespective of the duration of fever. In contrast, for persons ≥5 years of age, fever had to be present for 3 days before a blood culture was taken.

specimens will yield the organism in most carriers (>80%); chronic carriers are persons who shed *S.* Typhi in the stool for more than 1 year. Exposed individuals who are in the incubation period before illness can also have positive stool cultures.[45] Culture of bile-stained duodenal fluid by means of string capsule devices (Enterotest, HEDECO, Mountain View, CA) can often confirm the biliary carrier state in epidemiologically incriminated individuals who have negative stool cultures.[46] Alternatively, administering a laxative to individuals whose stool cultures are initially negative, leading to passage of one or more loose stools, often results in the cultures then becoming positive. The explanation for this increased stool culture sensitivity is that consequent to the laxative, typhoid bacilli from the bile are now rapidly excreted, having little exposure to the hostile environment created by the normal flora of the large intestine.

Systematic collection of blood cultures, in particular prior to and during efficacy trials of vaccines, has established the incidence of confirmed typhoid in many populations (Table 1).[47–55] Often these surveys yield rates that are much higher than were predicted, particularly in pre-schoolchildren.[32] Systematic surveys in some endemic areas demonstrated a surprising frequency of bacteremic typhoid infection among infants and toddlers[31,36,56]; in Chile and Peru these infant bacteremias due to *S.* Typhi were associated with only mild, self-limited febrile illness.[31,56]

In some other areas where bacteremia in febrile infants was systematically studied, the isolation of *S.* Typhi was rare in infants <12 months of age.[32,34,35,37,57] Elevated titers of serum IgG antibodies directed at the capsular Vi polysaccharide antigen that covers *S.* Typhi are encountered in >80% of chronic biliary carriers of *S.* Typhi but only rarely in healthy individuals[40]; <20% of persons with acute typhoid fever manifest elevated Vi titers. Therefore, the measurement of Vi antibodies has been repeatedly utilized with success as a screening test in nonendemic and endemic areas to detect chronic carriers.[24,38–42,58]

Serum H antibodies are IgG and long lived. However, they may be the consequences of stimulation by typhoid vaccination or by contact with other serovars of *Salmonella* that express the same Phase 1 flagellar antigen (d) as Typhi but have distinct O antigens.[43] Therefore caution must be exercised in selecting the populations to be sampled and in interpreting the results of serosurveys. Nevertheless, seroepidemiological surveys in which antibodies to *S.* Typhi flagellar antigens (H) are measured in different age groups have proven quite useful in comparing the prevalence of *S.* Typhi infection in different populations of interest and in estimating the age of peak infection.[43,44]

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organism. Confirmation of the diagnosis of typhoid fever requires recovery of *S.* Typhi from a suitable clinical specimen. Because of their practicality and relative ease of access, multiple blood cultures should be obtained from patients in whom the diagnosis is suspected clinically. The rate of recovery of *S.* Typhi in blood cultures depends on many factors, including the volume cultured, the ratio of the volume of blood to the volume of culture broth in which it is inoculated (ideally, the ratio should be ≥ 1:8), inclusion of anticomplementary substances in the medium (such as sodium polyanethol sulfonate or bile), and whether the patient has already received antibiotics to which the *S.* Typhi is sensitive. If three 5 ml blood cultures are obtained, *S.* Typhi can be recovered from the blood in approximately 70% of suspect cases.

The current "gold standard" of bacteriological confirmation of typhoid fever is the bone marrow culture, which is positive in 85–95% of cases, even when the patient has received antibiotics.[59–62] The use of duodenal string devices to obtain bile-stained duodenal fluid for culture is also quite useful. The combination of a duodenal string and two blood cultures generally provides a sensitivity of bacteriological confirmation equal to that achieved with bone marrow cultures, but without the invasiveness of the latter.[60] The bacteriological culture of skin snips from rose spots also provides a high yield.[59] Stool cultures generally lead to recovery of the organism in only 45–65% of cases. The yield tends to be somewhat higher in children.

S. Typhi does not ferment lactose; it produces H_2S but does not produce gas. As a consequence, suspicious colonies are evident on usual lactose-containing media as lactose-negative colonies. The biochemical pattern in triple sugar iron agar is rather characteristic, manifested by an acid butt without gas, an alkaline slant, and obvious H_2S production. Fresh isolates typically agglutinate with Vi but not group D antiserum. However, if the bacteria are boiled to remove the Vi capsule, a reaction with group D antiserum is then readily seen. The Vi antigen is an important epidemiological aid, since its susceptibility to lysis by bacteriophages allows for the classification of 80 definite and stable varieties of typhoid bacilli. This can help identify the source and spread of a specific strain.[63] The CDC is the principal source of phage typing in the United States. A more modern, very useful, molecular epidemiological tool for comparing isolates is the analysis of pulsed field gel electropherotypes of isolates.[64–68]

Polymerase chain reaction (PCR) methods, in theory, can be applied to clinical specimens to expedite a microbiological diagnosis of *S.* Typhi infection.[69,70] However, heretofore these methods have been amenable only to research and reference laboratories, since they are impractical for routine use in most laboratories in endemic areas that support clinical facilities. Moreover, significant hurdles will have to be overcome to adapt them to become inexpensive, simple point-of-care tests for use in clinical situations. In contrast, PCR methods are becoming increasingly attractive and practical to serotype *Salmonella* strains once they have been isolated from blood and other cultures.[71]

3.4.2. Serological Diagnostic Methods. Serodiagnosis of typhoid fever has been pursued since the late nineteenth century (1896) when Widal and Sicard[72] showed that the serum of patients with typhoid fever agglutinated typhoid bacilli. The Widal test, still practiced today in many areas, involves the search for agglutinins in the patient's serum. Whereas the test may be performed with antigen in tubes or on slides, the former is generally more accurate. By careful choice of antigen, both O and H antibodies can be selectively measured. By use of *S.* Typhi strain O901, which lacks flagellar and Vi antigens, *S.* Typhi O antibody can be selectively measured. To detect antibodies against the appropriate H antigen (d), a strain such as *S.* Virginia is selected that possesses the identical Phase 1 flagellar antigen (d) as *S.* Typhi but shares no O somatic antigens with serovar Typhi.[43] Most patients with typhoid fever have elevated levels of O and H

antibody at the time of onset of clinical illness.[43] Anderson and Gunnell[73] emphasized the importance and usefulness of H titers in serodiagnosis of typhoid fever. However, in general, the prevalence of H antibodies in adults living in endemic areas is too high for the test to be useful in that age group.[43,44] Nevertheless, it can be helpful in children less than 10 years of age in endemic areas and in persons of any age from nonendemic areas.[43] A history of inoculation with parenteral killed whole cell vaccines invalidates the use of the Widal test. One study from Indonesia supported the use of the slide test for O agglutinins of *S.* Typhi, even for adults in that endemic area.[74]

Serological tests that measure Vi antibody by passive hemagglutination or enzyme-linked immunosorbant assays (ELISAs), using highly purified Vi antigen, are excellent to screen for chronic *S.* Typhi carriers, most of whom have highly elevated levels of Vi antibody.[24,38–42,58] In contrast, Vi antibody tests are of little help in diagnosing acute typhoid fever because only a minority of patients with acute infection have detectable Vi antibody.[38,40]

3.4.3. Rapid Immunoassays. Over the years many attempts have been made to develop tests that detect *S.* Typhi antigens in blood, urine, or body fluids, thereby providing a rapid diagnostic test for typhoid fever. With few exceptions these tests have been disappointing and have failed to warrant the enthusiasm of the initial reports. Most of these assays are based on the detection of the O or Vi antigens of *S.* Typhi in blood or urine using coagglutination, ELISA, or countercurrent immunoelectrophoresis. Two other tests, TyphiDot and TUBEX, that detect antibodies to *S.* Typhi are currently under evaluation.[75–78] Although neither test is completely satisfactory, in some venues results have been guardedly promising.[75–78]

4. Biological Characteristics of the Organism

The taxonomy of *Salmonella* has changed repeatedly during the past 30 years, leading to considerable confusion.[15] Most authorities presently use the term, *S.* Typhi, to refer to the serovar that is the causative organism of typhoid fever. Since it is now considered a serovar and not a separate species, Typhi is written non-italicized and with a capital T. Serologically, *S.* Typhi falls into group D *Salmonella* on the basis of its O antigens 9 and 12, with 9 being the immunodominant epitope diagnostic of Group D. *S.* Typhi is motile and its peritrichous flagellae bear flagellar (H) antigen d, which is also encountered in approximately 80 other bioserotypes of *Salmonella*. A few percent of isolates from Indonesia have flagellae that bear other antigens including (j) (which results from an internal deletion within the *fliC* gene encoding (d))[79] and z66 (which may be found in conjunction with (d) or (j)).[80] Strains freshly isolated from patients possess on their surface an acidic polysaccharide capsule, the Vi (for virulence) antigen.[81] Vi prevents O antibody from binding to the O antigen and inhibits the C3 component of complement from fixing to the surface of *S.* Typhi.

S. Typhi exhibits a remarkable degree of homogeneity, rarely showing biochemical or serological variability. Exceptional are the few percent of isolates from Indonesia that bear phase 1 flagellar antigen j rather than d. Some Indonesian isolates express flagellar antigen z66, in addition to d or j. In practical terms, phage typing, using Vi phages, offers a reasonable tool that is often helpful in differentiating strains from different geographic areas.[39] Molecular epidemiological techniques such a multi-locus sequence typing that are useful with other bacteria are not helpful in differentiating *S.* Typhi isolates but rather corroborate the homogeneity of *S.* Typhi strains from diverse areas and time periods.[82] Similarly, analysis of the complete genome of two *S.* Typhi strains shows an impressive level of homogeneity.[83,84] PFGE is one molecular typing method that has proven useful for studying the epidemiology of *S.* Typhi.[64,68,85,86]

Typhoid bacilli are able to achieve an intracellular habitat promptly after oral ingestion. This property may account for the prolonged duration of disease in untreated patients and the relatively slow response to antibiotic therapy. Within macrophages of the reticuloendothelial system, the organism appears to be protected from the host's humoral immune mechanisms, and in this intracellular niche it also resists the effect of many antibiotics.

Like other gram-negative bacilli, the outer portion of the cell wall of typhoid bacilli consists of lipopolysaccharide (LPS) or endotoxin. This substance, which has biological properties that are important in the pathogenesis of typhoid fever, consists of three layers, the outermost being made up of a repeating sequence of sugars that determines the specificity of the O antigen. For typhoid, there are two O antigens, immunodominant epitope 9 and minor epitope 12. Rough mutant strains that lack the outer layer of LPS are nonpathogenic. The middle LPS layer is the R core. The inner layer of LPS is the lipid A endotoxin moiety. Endotoxin is pyrogenic and triggers the complement and clotting cascades; it also stimulates the release of cytokines that are deemed responsible for many of the clinical features of typhoid fever.

A curious feature of *S.* Typhi is that, in contrast with many other pathogenic enterobacteriaceae (e.g., *Shigella*, nontyphoidal *Salmonella*) that readily accept and stably carry R factor plasmids encoding antibiotic resistance, until $\sim$ 1990 this was the exception rather than the rule

with typhoid bacilli. Chloramphenicol, the first antibiotic used to treat typhoid fever, held a position of prominence for 25 years and is still important in less-developed countries where prevalent strains of *S.* Typhi remain susceptible. Then, rather abruptly, large-scale epidemics of chloramphenicol-resistant typhoid fever occurred, first in Mexico (1972)[87,88] and then in Southeast Asia (1974)[89]; sporadic cases of resistance were reported from the Middle East and India. Curiously, after approximately 2 years in Mexico, the resistant strain disappeared and was replaced by chloramphenicol-sensitive strains. Oral amoxicillin and trimethoprim–sulfamethoxazole were shown to be satisfactory alternatives for treating patients with chloramphenicol-resistant typhoid fever.[88] In 1979 and 1980, a similar abrupt appearance of chloramphenicol-resistant typhoid occurred in Lima, Peru;[90] these resistant strains also disappeared after a few years and were again replaced by chloramphenicol-sensitive *S.* Typhi. It is notable that in each of these instances the antibiotic-resistance genes were encoded on plasmids of incompatibility group H1.[87,90] A plausible biological explanation has never been found for the sudden appearance and regression of *S.* Typhi carrying these plasmids.

Beginning circa 1989, strains of *S.* Typhi resistant to chloramphenicol, amoxicillin, and trimethoprim–sulfamethoxazole disseminated widely throughout Asia, constituting a public health calamity until several years into the millennium.[9–11] Alternative effective antibiotics, including oral ciprofloxacin and parenteral ceftriaxone, are expensive and have other drawbacks. Moreover, the widespread use of ciprofloxacin and other fluoroquinolones, often in inadequate dosages and duration, selected for the emergence of fluoroquinolone-resistant strains.[12]

One other relevant biological property of *S.* Typhi is its ability to survive for a relatively long time in certain environmental niches, as in snow and surface waters, and in some foods. In some circumstances, typhoid bacilli may survive for several months, a property that has resulted in some "unexpected" epidemics because no recent source for the contamination was found.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

5.1.1. In the United States. Figure 1 shows the annual incidence rate of reported cases of typhoid fever in the United States per 10^5 population (data include Hawaii and Alaska, even before statehood) from 1950 to 2006. There was a leveling of the curve in the mid-1960s at about 2.0 cases/10^5 population and that incidence continued until the late 1990s. The spike in 1973 was due to the large waterborne outbreak that occurred in Dade County, Florida.[29,91] In that outbreak, the water supply in a migrant labor camp became contaminated and 222 cases of typhoid fever ensued. In the period 1989–1993, there were, on average, 473 cases of typhoid fever reported annually (range, 414–552),[92] whereas in the period 1994–2006, the average annual number of reported cases was 363 (range, 321–441).

S. Typhi infections in the United States were reviewed by the CDC for the period 1975–1984, representing a decade of experience beginning shortly after the Dade County outbreak;[93] 4,641 cases were reported to the MMWR during this period. More detailed epidemiological information was available for 2,666 of these cases for whom CDC special questionnaire forms were completed (57% of the cases). Twenty-nine deaths were reported, including 21 among domestically acquired cases and 8 among returned travelers. Mortality was five times higher for domestically acquired cases than for foreign travel-acquired cases (2.5 versus 0.5%). Among the domestically acquired cases, 9% were related to contact with a previously diagnosed typhoid carrier and 21% were related to contact with a newly diagnosed carrier. Although overall the majority of cases acquired domestically in the United States are single cases, 28% (266 of 934) of cases were related to outbreaks. During this decade of surveillance experience, there were three major outbreaks, all food-borne.

Travel-related cases of typhoid fever have increased notably since 1967,[4,5,94–96] so that by the time of the CDC review for the period 1975 through 1984,[93] 1,653 of the 2,666 reported cases (62%) were acquired during foreign travel. The median age for the 1,371 travel-related cases was 23 years, whereas that of the 857 cases acquired in the United States was 20 years. Two subsequent reports from the CDC that analyze typhoid fever cases among US citizens in the periods 1985–1994[4] and 1994–1999[5] also emphasize the importance of foreign travel. In the period 1985–1994, 72% of US cases of typhoid fever were associated with foreign travel and visits to six countries accounted for 80% of the travel-associated cases (Mexico, 28%; India, 25%; the Philippines, 10%; Pakistan, 8%; El Salvador, 5%; and Haiti, 4%). Coincident with the pandemic of multiple antibiotic-resistant typhoid fever in South Asia that began in 1989 and extended through the 1990s, the risk of developing typhoid fever among travelers to the Indian subcontinent was impressively high during the period 1990–1994 and exceeded 1,000/10^6 travelers; this is comparable to incidences observed in populations living in areas of moderate typhoid endemicity. Notably, the risk of developing typhoid fever in these travelers was usually associated with exposure lasting less than one month.

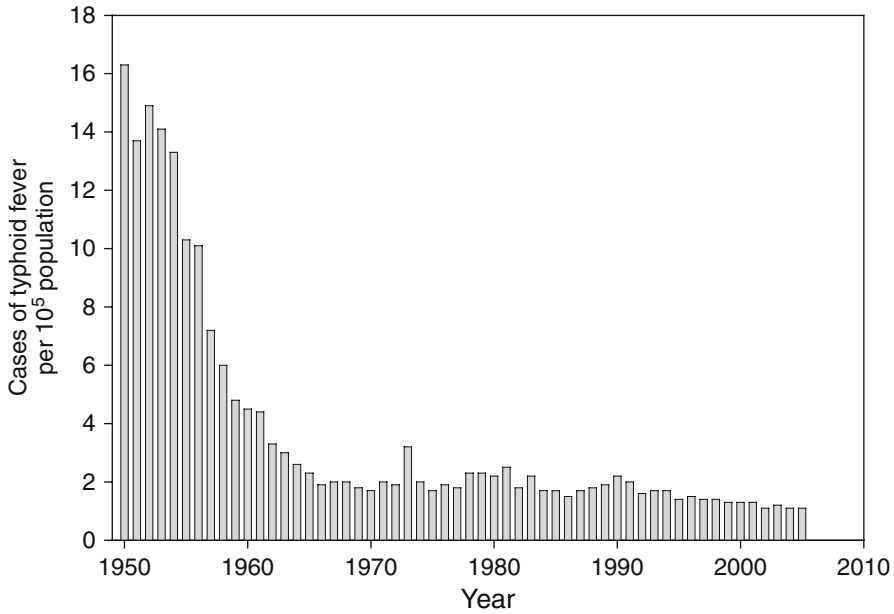


Figure 1. Typhoid fever by year, United States, 1950–2006.

In the period 1994–1999, 1027 of 1,393 (74%) US cases of typhoid fever were associated with foreign travel. Once again, travel to six countries accounted for 76% of the travel-associated cases.[5] During this period, travel to the Indian subcontinent represented the greatest risk (Table 2). Among patients who indicated a single reason for their travel, 80% were visiting family in the foreign country, whereas only 3% were tourists and 1% were on business-related travel.[5] Reviewing the CDC data from travel-associated cases of typhoid fever, Steinberg et al. report that among 626 travelers to a single country who reported their duration of stay, 16% were in the foreign country for < 2 weeks and 37% for less than 4 weeks.[5]

5.1.2. Worldwide. The WHO records the incidences sent to it by cooperating countries. These figures indicate that typhoid remains a major public health problem in South and Southeast Asia, the Middle East, Northeast Africa, sub-Sahara Africa, Central America, some parts of the Caribbean, and a few areas of South America. In endemic areas notification data appear to be a gross underestimation due to

Table 2. The Incidence of Typhoid Fever in US Travelers (Citizens and Residents) to Various Regions of the World, 1982–1994

	1982–1984[a]		1985[b]		1994[b]		1994–1999[c]
Country visited	Mean annual no. of cases	No. cases per 10^6 travelers	Annual no. of cases	No. cases per 10^6 travelers	Annual no. of cases	No. cases per 10^6 travelers	Mean annual no. of cases
Mexico	77	20.2	33	1.9	34	1.3	23
Haiti	3	41.8	–	–	–	–	9
Peru	10	173.8	–	–	–	–	
Chile	3	58.4	–	–	–	–	
India	26	118.5	–	–	–	–	56
Pakistan	6	105.1	–	–	–	–	24
Indian subcontinent[d]	–	–	18	234	54	812	–
Indian subcontinent[e]	–	–	–	–	–	–	100

[a]Data from Ryan et al.[93]
[b]Data from Mermin et al.[4]
[c]Data from Steinberg et al.[5]
[d]India, Pakistan, Bangladesh, Sri Lanka, and Myanmar.
[e]India, Pakistan, Bangladesh, Sri Lanka, Afhanistan, Nepal, Bhutan, and the Maldives.

incomplete reporting and insufficient means for bacteriological confirmation. However, it is difficult to quantitate the true magnitude of the typhoid fever problem worldwide because the clinical picture is confused with many other febrile infections and the capacity for bacteriological confirmation is absent in most areas of the less-developed world.[97,98] Nevertheless, the most recent exercise to quantify the global morbidity and mortality burden of enteric fever estimates that each year there occur circa 21.6 million cases of typhoid fever and 216,510 deaths; it is also estimated that there occur $\sim$ 5.4 million cases of paratyphoid fever.[98]

Population-based surveillance for enteric fever undertaken by the systematic collection of blood cultures from febrile patients provides hard data on the incidence of typhoid and paratyphoid fever in different populations, age groups, and geographic areas (Table 1). However, several factors affect the sensitivity of such surveillance. These factors include whether the surveillance is passive, active, "modified passive", or "modified active"; the volume of blood collected and the antibiotic treatment practices. By passive surveillance is meant the fact that febrile cases are detected when the patient seeks health care at a treatment facility. "Modified passive" indicates that special healthcare facilities were introduced specifically to bolster the existing system and increase the access of febrile patients to health care to enhance surveillance. Active surveillance implies that investigators visit households at a certain frequency (e.g., 1–2 times per week) to take temperatures of subjects; the febrile subjects then either have blood cultures collected in the household or the febrile subjects are sent to a nearby health facility where they are cultured. "Modified active" surveillance means that household visits are made to detect patients with fever but that instead of either drawing blood cultures or arranging for the subject to go to a health facility for collection of a blood culture the subject is merely "encouraged" to go to a facility to have a blood culture. Over the past 25 years there have been multiple studies that have involved blood culture-based surveillance of enteric fever, either to assess the population disease burden or as part of controlled field trials to measure the efficacy of vaccines. Results of these studies are shown in Table 1 with the data adjusted to represent annual incidence rates.

Surveillance data generated by quantitating the incidence of bacteriologically confirmed typhoid fever in placebo groups participating in large-scale field trials of typhoid vaccines[47,49–54] or in preparation for undertaking vaccine field trials[35,37] show annual incidence rates from 103 to $>$1,000 cases per 10^5 population in typhoid-endemic areas (Table 1). Several major assumptions or "corrections" must be made if one is intending to extrapolate from local data that involve unusually intense surveillance methods in order to estimate the disease incidence and burden for larger populations within which the surveillance populations lie (e.g., entire country or regions of continents). It is widely agreed that data from such local surveillance in fact underestimate the overall enteric fever burden. Among the factors believed to result in the underestimate are that many patients will have received antibiotics prior to having a blood culture, resulting in a false-negative culture. Also, patients with milder forms of enteric fever may be treated with antibiotics as ambulatory patients and never have a blood culture, thereby constituting undetected cases. On the other hand, intensive surveillance studies in relation to vaccine field trials or vaccine impact projects typically take place in populations in which it is already known that typhoid fever and paratyphoid fever exist ("hot spots"), although precise quantification of the incidence may not be available before special surveillance studies are introduced. Since the prevalence of *S*. Typhi and *S*. Paratyphi are geographically spotty, one must be cautious in making extrapolations. To give one example, during the 1970s and 1980s, when typhoid and paratyphoid fever were hyper-endemic in Santiago, Chile, enteric fever was observed at a much lower incidence in most other parts of the country. Clinical observations, notification data and seroepidemiologic studies all corroborated this geographic difference in distribution of the disease.[44] Yet exercises to approximate the typhoid fever disease burden for all of South America extrapolated incidence data from Santiago field trial control groups to the population of the entire continent.

5.1.3. Prevalence of Carriers. The total number of typhoid carriers in the United States is not known. In 1962, the number was estimated to be 3,637 and in 1978 more than 2,000. In Arkansas, the rate of carriers was 3–4/10^5 population in 1978. Many of the known carriers are elderly, and with the low incidence of typhoid fever in this country, few new indigenous carriers will appear to replace those who will die in the next decade. However, the influx in recent years of immigrants from Central America and South and Southeast Asia undoubtedly includes some chronic typhoid carriers from these endemic areas. Nevertheless, in view of the well-developed sanitary conditions existent throughout the United States, there are no grave epidemiological consequences anticipated from the arrival of such carriers unless they become employed as food handlers.[24]

In the preantibiotic era in the United States, some predictions were made about the decline in numbers of carriers; these predictions have come true. The estimated carrier prevalences in 1935 for Massachusetts and Mississippi were 48 and 288/10^5 population, respectively. In New York, in 1940, there were an estimated 2,490 carriers, for a prevalence

of 41.8/100,000 population.[99] According to the 1943 report by Ames and Robins[99] of the New York State Department of Health, the number of carriers in New York would have fallen from 2,490 in the early 1940s to about 190 by 1980. Their assumptions in arriving at these figures did not predict the effect antibiotic therapy (unavailable in their time) would have on the development of and treatment of the carrier state. Nonetheless, in New York in 1980, the total number of known carriers was 175, remarkably close to the 190 predicted 37 years earlier by Ames and Robins.

Only a few quantitations have been made of the prevalence of chronic carriers in less-developed countries. One such survey carried out in Santiago, Chile, during a period when typhoid fever was highly endemic, estimated a crude prevalence of 694 carriers/10^5 population.[100] This high prevalence was related in part to the high prevalence of cholelithiasis in the adult female population of Santiago.[101]

5.1.4. Prevalence of Antibody. The prevalence of antibodies to O, H, and Vi antigens in US citizens in the late 1960s and early 1970s could be estimated from the extensive serological data accumulated in the course of a unique series of volunteer studies with wild-type *S.* Typhi conducted to evaluate the efficacy of typhoid vaccine.[45] Baseline antibody titers (obtained at time of entry into the study) revealed that older men had higher geometric mean H antibody titers than those 15–20 years their junior. The earlier the year of birth, the more likely a volunteer was to show an elevated titer of H antibody at the time of participation in the study, and if birth occurred after 1935, a period when the disease was rapidly disappearing in the United States, the influence of age disappeared. These values held whether or not there was a military history, i.e., an opportunity to have received vaccine. Since these studies investigated persons living in a nonendemic area, the percentages of volunteers lacking detectable O, H, and Vi gave an estimate of the lack of past exposure to *S.* Typhi antigens, whether by previous disease, vaccine administration, or exposure to other salmonellae-containing common and cross-reacting O, H, or Vi antigens. Thus, of 331 men, 52% had no detectable H, 74% no detectable O, and 42% no detectable Vi serum antibodies. The majority of the younger volunteers participating in the later phases of the study fall into these groups. Of those men who had demonstrable circulating antibody titers, only a small percentage had elevated values. If the trend noted in that study continued, one would expect an even smaller percentage of current US citizens to have detectable antibodies as opportunities for exposure to typhoid antigens in the United States continue to diminish. This hypothesis is predicated on the assumptions that (1) the sources of acquired disease will continue to decline, i.e., fewer carriers will develop and the present ones will die off; (2) there will be continued minimal utilization of typhoid vaccine in this country; (3) the number of immigrants to the United States from typhoid-endemic areas (e.g., South Asia, Haiti) will not be so large as to affect markedly the prevalence of antibodies; (4) there will be better control over other sources of salmonellae that may have cross-reacting antigens. More than 140 serovars of *Salmonella*, some of which commonly cause gastroenteritis, have O antigens that cross-react with the typhoid bacillus antigens in the Widal agglutination test. Similarly, there are at least 70 strains with cross-reacting H antigens, although few of these have been recognized in association with human clinical illness. These antigens may be the source of "typhoid" O and H antibodies detected by the Widal test in many US citizens today.

Antibody prevalence surveys have also been carried out in areas where typhoid is highly endemic. Seroepidemiological studies in Peru and Chile performed in the 1970s showed that by 15–19 years of age, 50–80% of teenagers had serological evidence of past infection with *S.* Typhi.[43,44]

5.2. Epidemic Behavior and Contagiousness

In the United States, scattered outbreaks of typhoid fever occur when a carrier contaminates food or a water supply.[24,25,29] The epidemic that occurred in Dade County, Florida illustrates how a segment of our society may become infected when sewage disposal and water supply are improperly controlled.[29] The critical epidemiological link in this outbreak was thought to be a mentally retarded child who acquired the infection from an asymptomatic carrier living in a neighboring house. It is believed that this child subsequently contaminated the flawed water supply in the migrant labor camp, resulting in 222 cases in the camp population, 184 of whom were hospitalized. This was the largest single outbreak in the past half century. Other large epidemics in industrialized countries have occurred in the past five decades in Scotland (504 cases)[102] and Zermatt, Switzerland (280 cases).[103] In Scotland, the source of the infection was canned corned beef shipped from Argentina. The meat per se did not contain typhoid bacilli prior to processing (raw meat has never been found to be contaminated with this organism, attesting to the unique adaptation of typhoid bacilli to humans). However, after the canning process, the cans were heat sterilized and then placed in contaminated river water to cool. As the cans cooled, water was drawn into the cans through minute leaks in the seams. Those who handled the meat from the cans became potential cases as well as possible disseminators of the bacteria. Furthermore, when the meat was cut by machines, the blades became contaminated, and other meats subsequently cut on these machines

also acquired typhoid bacilli, which was then spread to other consumers.[102]

The epidemic in Zermatt occurred from contamination of the town water supply. The contamination lasted for at least 1 month before the epidemic was recognized and chlorination and other measures could be instituted to treat the water.

Another large epidemic of interest was caused by chloramphenicol-resistant *S.* Typhi in Mexico City from 1972 to 1974.[88] At the time typhoid was endemic in that city, but the marked increase in number of typhoid fever cases admitted to hospitals quickly focused attention on the fact that this new strain was responsible for two thirds of the increased incidence. An epidemiological investigation suggested that the strain may have been disseminated by soft drink vehicles made with contaminated water.[104] The Mexican strain was found to be responsible for typhoid fever in Switzerland, the United Kingdom, the United States, and other parts of the world. Travelers to Mexico acquired the disease and brought it back to this country and the others. In Los Angeles in 1972, the incidence of the disease doubled, due mainly to imported cases from Mexico.[105] Fortunately, very few secondary cases of typhoid fever occurred in this country as a result of the importation of the antibiotic-resistant Mexican strain.

One other water source of infection that has been documented involves oysters harvested from polluted water; typhoid and other enteric pathogens can be concentrated from the water strained by the bivalves.[106] In situations where latrines are not fly-proofed or exposed human feces is common, flies have been shown to transmit typhoid bacilli by contamination of their feet as they feed on feces and subsequent carrying of the organisms to food.[107]

Typhoid fever is essentially spread only by consumption of contaminated food or water vehicles. Under rare circumstances, direct person-to-person transmission through anal–oral spread may occur, as among homosexuals who indulge in anolingual sexual practices.[108,109]

Transmission occurs among clinical microbiologists in hospital diagnostic bacteriology laboratories.[6,7] The handling of specimens containing *S.* Typhi has caused numerous cases of typhoid fever. Twenty-five cases of laboratory-acquired typhoid fever were reported to the CDC from 1977 to 1979, among which 14 were related to a proficiency-testing program. These data indicate the need for a good training program in order to upgrade the skills and techniques of microbiology technicians. The organism can be handled safely in hospital diagnostic laboratories, but great care must be taken to avoid contaminating fingers, pens, pencils, and laboratory paraphernalia, since these may serve as fomites to transmit the bacteria to the mouth. In some instances, it is believed that aerosols may be involved in the transmission of *S.* Typhi within clinical microbiology laboratories, not by direct inhalation but by contamination of nearby surfaces and objects that subsequently serve as fomites. Aerosols may be created as culture tubes containing *S.* Typhi are opened, leading to contamination of nearby objects, which subsequently contaminate hands. If hands are not adequately washed, they can carry the organisms to food or directly to the mouth. Small numbers of volunteers exposed to small-particle aerosols containing 10^5 typhoid bacilli failed to develop signs of infection. This dose given by mouth caused disease in about 38% of volunteers; thus, the respiratory tract appears to be a less effective route of inoculation than the gastrointestinal tract.

Typhoid fever has a relatively low risk of contagion. Person-to-person transmission can occur by anal–oral spread such as changing the soiled bed linens of an undiagnosed patient. Patients with typhoid fever shed the etiologic agent from the gut and urinary tract. Aerosol dissemination from these sources would appear to be highly unlikely.

5.3. Geographic Distribution

In less-developed areas of the world where fecal contamination of water and food is common, incidence rates of many enteric infections are high, including typhoid fever. Typhoid fever remains common in South, Southeast, and Central Asia, much of sub-Sahara Africa, the Philippines, Haiti, some regions of Central and South America, a few Eastern European countries, and parts of the Middle East.

Transmission of *S.* Typhi is favored wherever sanitation is primitive such that human fecal waste can contaminate untreated water sources that are widely consumed. Additional transmission of a more sporadic nature is favored where hygiene practices are primitive and chronic or short-term carriers can contaminate food. However, typhoid and paratyphoid fever are not common in every less-developed region. Rather, the prevalence of the disease is spotty and requires the presence of the human reservoir as well as the means for transmission. In most areas of the world where *S.* Typhi is prevalent the syndrome of classical clinical typhoid fever is most often observed in school-age children and young adults. In some densely populated urban slums the peak incidence of clinical typhoid is seen in preschoolchildren. In general overt typhoid fever is uncommon in infants less than 12 months of age (although some severe cases are occasionally seen in this age group).

In the latter half of the nineteenth century, high incidence rates of typhoid fever were observed in most of the major cities of Europe and North America. Transmission

of typhoid in these cities was amplified as untreated water from nearby rivers that had heavy fecal contamination was an important source of municipal drinking water for inhabitants of the cities. Treatment of the water sources led to striking decreases in the transmission of typhoid, reflected by plummeting incidence rates.[16,17] Typhoid fever is still common in a number of large cities in the less-developed world where segments of the population are not served by piped, treated, monitored water supplies. These cities include Cairo, Egypt; Jakarta, Indonesia; Bombay, New Delhi, and Kolkata, India; Dhaka, Bangladesh; Dushanbe, Tajikistan; and Karachi and Lahore, Pakistan.

In some large cities, endemic typhoid persisted despite provision of virtually all of the population with treated water. In such exceptions, another form of amplification was responsible. One example was Santiago, Chile in the 1970s and 1980s, where typhoid remained notably endemic despite 96% of the population having access to treated, bacteriologically monitored water.[44,110] Irrigation of crops of salad vegetables such as lettuce, celery, and cabbage during summer with untreated sewage was epidemiologically linked as the amplification event.[111,112] Abrupt discontinuation of this practice following a small outbreak of cholera in 1991 led to a precipitous decline in the incidence of typhoid fever and its virtual disappearance as a public health problem in an infamous endemic area where a few years earlier the WHO sponsored four successive large-scale field trials of efficacy of typhoid vaccines.[47–49,113,114]

5.4. Temporal Distribution

In endemic areas, typhoid typically exhibits seasonality but the peak season differs based on a number of epidemiologic features that affect transmission and survival of *S.* Typhi. In temperate and sub-tropical areas where typhoid remains endemic, most cases occur during the summer months. The cases acquired by US tourists usually occur in the summer, coinciding with the peak travel time. The remaining cases appear sporadically during the year. In many endemic areas, typhoid typically shows a highly seasonal pattern. In Chile, Ecuador, and Peru there was a clear-cut peak in summer,[110] while in Northern Nigeria[115,116] typhoid peaks during the dry season.

5.5. Age

Perhaps the most striking and fascinating epidemiological feature of typhoid fever in endemic areas is its age-specific incidence pattern. The classical age-specific epidemiologic pattern that has been described for decades from many disparate endemic areas is one in which the incidence of typhoid is low in the first year of life, begins to be seen in pre-schoolchildren, peaks in school-age children, remains high in young adults, and then falls again in middle age (Table 3). These age-specific incidence data were based on passive surveillance methods in which cases were identified among cases who felt ill enough to seek health care.[44,110,117,118] More recent passive surveillance studies from South Asia[37] and Southeast Asia[35] and Central Africa[36] based on detection of cases who seek health care have revealed similar age-specific incidence patterns in which the peak incidence of bacteriologically confirmed typhoid fever occurs in school-age children (Table 1).

In the early 1990s, Mahle and Levine[56] reviewed the validity of the dogma that typhoid fever is relatively rare in infants, toddlers, and pre-schoolchildren based on data available at that time. Notification data and hospital-based reports that they reviewed supported the classical teaching, demonstrating a much lower incidence in the 0- to 4-year-old age group than in school-age children (5- to 19-year-olds).

Table 3. The Mean Annual Age-Specific Incidence of Typhoid Fever per 100,000 Population, Based on Notification Data in Diverse Geographic Regions and Time Periods

Age group (years)	Palestine Mandate 1934–1942[118]	British Guiana 1956–1960[117]	Peru 1971[56]	Santiago, Chile 1977–1981[44]
0–4	127	48	67	89
5–9	307	180[a]	114	272
10–14	266	180[a]	144	333
15–19	236	112[a]	129	283
20–24	271	112[a]	102[a]	247
25–29	196	66[a]	102[a]	153[a]
30–34	108[b]	66[a]	50[c]	153[a]

[a]Incidence reported for cohorts spanning 10 years.
[b]Incidence of 30- to 39-year age group.
[c]Incidence of population >30 years of age.

Importantly, they examined age-specific incidence rates in a series of waterborne outbreaks and found a similar age distribution as was observed in endemic disease, suggesting that the lower morbidity in younger children is not due to decreased exposure to *S.* Typhi but rather to host factors. Mahle and Levine concluded that following exposure and infection most very young children (< age 2 years) appear to develop a mild form of clinical illness that is not readily recognized as typhoid fever. This conclusion was supported by systematic blood culture studies in typhoid-endemic areas among febrile children who were brought to health centers. *S.* Typhi bacteremia was documented in infants and toddlers < 24 months of age but they clinically manifested only mild febrile illness that was most often diagnosed as a "viral syndrome".[31] Young children with typhoid also exhibit less prominent pathology of the small intestine.[31] Together, these features suggest that young children readily become infected with *S.* Typhi, but that a distinct pathogenesis usually produces a remarkably mild illness.

However, a fresh look at the question of the age-specific incidence pattern of endemic typhoid fever has resulted from several studies carried out in South Asia since the mid-1990s that utilized active surveillance. The active surveillance methods involved house to house visits once or twice weekly to detect cases of fever among household members followed by the collection of blood cultures from the febrile subjects.[32,34] Whereas these studies have corroborated that typhoid fever is rare in infants < 12 months of age, these active surveillance studies revealed a high incidence of *S.* Typhi bacteremia in toddlers and pre-schoolchildren, 12–59 months of age.[32,34]

Older adults in endemic areas appear to be relatively immune, presumably because of frequent boostering of immunity acquired earlier. In the United States, those most likely to acquire the disease at home are adults under age 30.[93,94] Three fourths of 857 patients reported between 1975 and 1984 were in this age range. Median age was 20.[93] The travel-associated cases had a median age of 23 years. Persons of these ages had no background immunity, having been born and raised in a country where the disease is no longer prevalent ($\sim$1 case/10^5 annually since 1970 compared to $\sim$10 cases/10^5 in 1955 and about 30 cases/10^5 in 1945). Vaccine-induced immunity, including that conferred by the more recent Ty21a and Vi vaccines, is less effective in young children, presumably because of a lack of background immunity.

While the median age of persons with typhoid fever was 22–24 years in the period 1984–1986 in the United States, the median age of carriers in those years was 71, 58, and 69 years, respectively. In endemic areas, the prevalence of carriers steadily increases with age.

5.6. Sex

There is no prominent effect of sex on the incidence of acute typhoid fever; both sexes are equally susceptible. However, the prevalence of chronic carriers is threefold greater in females.[99,100] This is because the propensity to become a chronic carrier following acute typhoid infection, either symptomatic or subclinical, is related to the presence of preexistent gallbladder disease. Infection of the biliary tree and the gallbladder is universal during acute typhoid fever. Indeed, culture of bile-stained duodenal fluid specimens is one of the highest yield clinical bacteriological procedures to isolate *S.* Typhi during acute typhoid fever.[60] Just as the prevalence of cholelithiasis and chronic gallbladder disease occurs with greater frequency in females and increases with age, so too an identical prevalence pattern of chronic biliary typhoid carriers is observed in endemic areas.[100]

5.7. Race

There are no data to support racial susceptibility to typhoid fever. While many series in the US literature indicate that more blacks have typhoid fever, it is probable that the living conditions, e.g., poor sanitary conditions, are the precipitating factor, not race. In volunteer studies, no evidence of race-associated susceptibility was detected. An unusually virulent form of clinical typhoid fever used to be encountered with frequency in Jakarta, Indonesia, where obtundation and shock were common.[61,119] The case fatality ratio in such severely ill patients was about 50% unless steroids were coadministered with appropriate antibiotics. However, it remained around 10% among the most ill hospitalized patients, even with adjunct steroid therapy.[119] Multiple hypotheses have been put forth to explain this severe form of typhoid fever, which is concentrated on the island of Java. One hypothesis proposes that the genetic makeup of the Javanese population predisposes them to respond to typhoid infection with a particularly severe clinical syndrome. Indeed, recent reports that describe enhanced susceptibility and resistance to typhoid fever in Vietnamese subjects based on certain genotypes give credence to the role of genetics in influencing risk of developing clinical typhoid fever following exposure and the severity of disease.[121–123]

5.8. Occupation

Several occupations have historically been associated with an increased risk for acquiring typhoid fever. These include sewer workers, soldiers,[124,125] and most recently, clinical microbiologists.[6,7] Clinical microbiologists are now

recognized as one high-risk US population for which immunization with one of the new vaccines is indicated.

5.9. Occurrence in Different Settings

In industrialized countries, a large proportion of sporadic cases occur among travelers (Table 2).[5,126–130] When outbreaks occur in the United States and other industrialized countries that have high levels of sanitation, controlled water supplies, and sophisticated food hygiene practices, a careful epidemiological investigation almost always incriminates the food or water vehicle responsible for the outbreak and often identifies a chronic carrier responsible for its contamination.[24,25,29] Similarly, epidemiological investigation of sporadic, non-travel-associated cases in the United States also often identifies a chronic carrier, such as an elderly family member, who serves as the reservoir of infection.[131]

In contrast, in typhoid-endemic areas in less-developed countries, where populations live in underprivileged conditions without controlled, piped water supplies, means to remove human waste, refrigeration for preserving food, or adequate food hygiene practices, it is usually exceedingly difficult to identify the specific vehicles of transmission of sporadic cases or the human reservoir.[132–136]

5.10. Socioeconomic Factors

Wherever inadequate sanitary facilities exist and water supplies are subject to fecal contamination, the opportunity exists for enhanced transmission of all enteric infections, but especially typhoid fever. The unique adaptation of *S.* Typhi to humans and the survival characteristics of this organism make it a particular threat to spread in populations living under substandard sanitary conditions. Prior to the availability of treated water and sewer systems, all strata of society were at risk. Endemic regions continue to exist in the world primarily because the poorer segments of the population in those areas remain underserved by adequate water supply and sanitation services and lack of detection and restriction of carriers. Provision of means to remove human waste and of treated, bacteriologically monitored water precludes widespread transmission and virtually eliminates typhoid fever from previously endemic loci.

5.11. Other Factors

There is no reliable evidence indicating increased or decreased susceptibility of patients with malnutrition to typhoid fever. The untreated disease causes a severe metabolic drain, so illness in a host with undernutrition will create a double hazard. Patients with various parasitic infections such as roundworms or schistosomes are readily infected with *S.* Typhi. In the first instance, typhoid fever in the host will cause the roundworms to migrate out through the mouth and anus. Reasons for this exodus from the gut are unknown. Schistosomiasis causes tissue scarring and obstruction, especially in the genitourinary tract by *Schistosoma haematobium*, and *S.* Typhi can remain indefinitely in the scar tissue or adherent to the trematode itself.[137] Salmonellae express fimbriae that allow adherence to the glycolipids on the surface of the worm.[137] A common consequence of such symbiotic coinfection is recurrent silent *S.* Typhi bacteremia in the host without any clinical evidence of typhoid fever.

6. Mechanisms and Routes of Transmission

Typhoid bacilli enter the host by the oral route via ingestion of contaminated food or water. The disease has been shown to be one of several enteric infections that can be sexually transmitted by anolingual sexual practices.[108,109] Flies can carry *S.* Typhi on their feet and have been incriminated, indirectly, as mechanical vectors that contaminate food.[107] Rarely, infection is believed to have been acquired by aerosol.

It is helpful to consider that typhoid fever is transmitted by the fecal–oral route in two broad manners: a "short cycle" and a "long cycle." The short cycle is typified by an individual carrier who contaminates food vehicles that are consumed by family members, or by participants at a communal gathering (wedding, party, etc.), or by a chronic carrier food working as a food handler in a restaurant.[24] Thus, it is a short distance from point of contamination to consumption of the vehicle by the susceptibles. In contrast, examples of long-cycle infection include contamination of water supplies by sewage,[26] contamination of piped water supplies that are distributed,[26,138] and finally widespread dissemination of typhoid bacilli via contaminated foods that may be transported over long distances.[102]

Examples of short-cycle sporadic cases and outbreaks include the families served by the infamous cook, "Typhoid Mary,"[139] and restaurant outbreaks in Texas,[140] Maryland,[24] and New York.[141] Most outbreaks in the United States are related to the short-cycle mode of transmission.[24,91,141]

The long-cycle transmission is exemplified by the Aberdeen[102] and Zermatt[103] epidemics of the 1960s and the Northern Israel outbreak in the late 1980s.[142] The Aberdeen epidemic was caused by consumption of contaminated tins of corned beef canned in Argentina and shipped

to Scotland. The Zermatt epidemic was caused by a carrier contaminating a mountain stream at the point where it served as the intake for the water supply of Zermatt.

7. Pathogenesis and Immunity

7.1. Pathogenesis

S. Typhi and *S.* Paratyphi A and B are highly invasive bacteria that rapidly and efficiently pass through the intestinal mucosa of humans to eventually reach the reticuloendothelial system, where, after a 8- to 14-day incubation period, they precipitate a systemic illness. *S.* Typhi is a highly host-adapted pathogen; humans comprise the only natural host and reservoir of this infection. Our comprehension of the steps involved in the pathogenesis of typhoid fever comes from four sources: (1) clinicopathological observations in humans[143]; (2) volunteer studies[45]; (3) studies of a chimpanzee model[144]; and (4) analogies drawn from *S.* Typhimurium and *S.* Enteriditis infection in mice – the "mouse typhoid" model.[145] The probable steps in the pathogenesis of *S.* Typhi infection in humans are summarized below.

Susceptible human hosts ingest the causative organisms in contaminated food and water. The inoculum size and the type of vehicle in which it is ingested greatly influence the attack rate for typhoid fever and also affect the incubation period, a relationship that was documented in volunteer studies in the 1960s (Table 4). Doses of 10^9 and 10^8 pathogenic *S.* Typhi ingested by volunteers in 45 ml of skim milk induced clinical illness in 98% and 89% of individuals, respectively; doses of 10^5 caused typhoid fever in 28–55% of volunteers, whereas none of 14 subjects who ingested 10^3 organisms developed clinical illness.[45]

In the fasting normochlorhydric stomach, gastric acid undoubtedly inactivates many of the typhoid bacilli that are ingested. However, some foods may serve as a buffer to diminish this otherwise formidable protective acid barrier. After passing through the pylorus and reaching the small intestine, the bacilli rapidly penetrate the mucosa by one of two mechanisms to arrive in the lamina propria. One mechanism involves uptake of the bacilli into endocytic vacuoles and their passage through the enterocytes to be ultimately released into the lamina propria without destroying the enterocyte.[146] In the second, quite distinct, invasive mechanism, typhoid bacilli are actively taken up by M cells,[147] the domelike epithelial cells that cover Peyer's patches and other organized lymphoid tissue of the gut. From here they enter the underlying lymphoid cells. In yet a third mode of entry, it has been proposed that *Salmonella* may sometimes take a paracellular route between enterocytes.[148]

Upon reaching the lamina propria in the nonimmune host, typhoid bacilli elicit an influx of macrophages and dendritic cells that ingest the organisms but are generally unable to kill them. Some bacilli apparently remain within macrophages of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where further multiplication and ingestion by macrophages take place. Eventually, there is a release of tumor necrosis factor-alpha, interleukin-2 (IL-2), IL-6, and other inflammatory cytokines by the macrophages (and perhaps by enterocytes).

Postmortem studies have documented the marked inflammatory responses that occur in the distal ileum in the Peyer's patches and other organized lymphoid aggregations. Presumably, cytokine release elicited by the intracellular *S.* Typhi is responsible for these cellular changes. It is from these lesions in the distal ileum and ascending colon that hemorrhage can occur later in the disease course. Gross bleeding comes from eroded vessels in or near the Peyer's patches. This complication occurs late in the second week in about 1–3% of untreated patients and rarely in patients whose treatment with an appropriate antibiotic is begun late in their clinical course. Perforations of the bowel wall occur in the same sections of the gut as the hemorrhages.

Shortly after invasion of the intestinal mucosa, a primary bacteremia is believed to take place in which *S.* Typhi are filtered from the circulation by fixed phagocytic cells of the reticuloendothelial system. The main route by which typhoid bacilli reach the bloodstream in this early stage is by lymph drainage from mesenteric nodes entering the thoracic duct and then the general circulation. Conceivably, ingestion of a massive inoculum followed by widespread invasion of the intestinal mucosa could result in rapid and direct invasion of the bloodstream. As a result of this primary bacteremia, the pathogen rapidly attains an intracellular haven throughout the organs of the reticuloendothelial system where it resides during the incubation period (usually 8–14 days) until the onset of clinical typhoid fever. Clinical illness is

Table 4. Incubation Periods of Typhoid Fever Related to Size of Infecting Dose[a]

Dose[b]	Attack rate (%)	Mean incubation period (days)
10^3	0	–
10^5	38	13
10^7	50	11
10^9	90	5.8

[a]Data from Hornick et al.[45]
[b]Colony-forming units.

accompanied by a fairly sustained, albeit low level (1–10 organisms/ml), "secondary" bacteremia.

The Vi antigen is a virulence property and in most endemic areas and outbreaks virtually all strains freshly isolated from patients possess this polysaccharide capsule.[81] Both epidemiological observations and studies in volunteers support the contention that *S.* Typhi strains that possess Vi are more virulent than strains lacking this polysaccharide.[45]

The bacteremia of typhoid fever will persist for several weeks if antibiotic therapy is not given, and the blood may be seeded from the liver as well as the lesions in the intestinal tract. The symptoms and signs of typhoid fever are not believed be due to circulating endotoxin. Most patients have no serum endotoxin detected by the limulus assay.[149] However, in patients who present with septic shock syndrome, circulating endotoxin can be demonstrated.[119] These patients have a higher mortality rate and require a combination therapy of steroids and antibiotics.[119]

Relapses were observed in about 8% of patients suffering from typhoid fever in the preantibiotic era. The rate in patients treated with the first (chloramphenicol) and second (ampicillin, amoxicillin, and trimethoprim/sulfamethoxazole) generation of antibiotics used for therapy of typhoid ranged from 10 to 35%. The organisms that initiate relapse appear to come from within the reticuloendothelial system. Typhoid bacilli can be isolated from liver biopsies during the relapse, and also from bone marrow many months after the patient has fully recovered from the symptoms. Relapses usually occur about 3 weeks after the last febrile day or about 2 weeks after cessation of antibiotics. Most relapses are milder than the initial illness, last for only a few days, and respond promptly to appropriate antibiotic therapy. Relapse after treatment with oral fluoroquinolones and azithromycin and after parenteral ceftriaxone therapy is distinctly uncommon.

During the primary bacteremia that follows ingestion of typhoid bacilli and seeds the reticuloendothelial system, organisms also reach the gallbladder, an organ for which *S.* Typhi has a remarkable predilection.[144,150] *S.* Typhi can be readily cultured from bile or from bile-stained duodenal fluid in patients with acute typhoid fever.[60,61,120] In approximately 2–5% of patients, the gallbladder infection becomes chronic.[99] The propensity to become a chronic carrier is greater in females and increases with age at the time of acute *S.* Typhi infection, thereby resembling the epidemiology of gallbladder disease.[99,101] The infection tends to become chronic in those individuals who have preexistent gallbladder pathology at the time of acute *S.* Typhi infection. Carriers shed as many as 10^9 organisms/g feces.[151] These organisms are propelled the length of the gastrointestinal tract without penetrating and causing disease in the host.

7.2. Immunity

Age-specific incidence patterns suggest that repeated exposure in endemic areas results in the acquisition of immunity. Moreover, it is well recognized that chronic carriers do not themselves develop clinical typhoid fever, despite having hundreds of billions of virulent typhoid bacilli pass the length of their intestine each day. On the other hand, natural infection-derived protective immunity is not absolute. In volunteer rechallenge studies, an initial clinical typhoid infection conferred only 30% protection against subsequent challenge with the homologous *S.* Typhi strain.[152] Similarly, repetitive clinical infections have been recorded in closed populations that experienced multiple sequential outbreaks of typhoid fever.[124]

A broad immune response is evident following wild-type typhoid infection, with the appearance of serum antibodies, mucosal SIgA responses, an array of cell-mediated immune responses, and a form of antibody-dependent cellular killing of *S.* Typhi. The licensed parenteral purified Vi polysaccharide and attenuated Ty21a live oral vaccines each elicit widely divergent immune responses, yet in its own way each confers significant protection. This suggests that several of the immune responses elicited by wild-type infection or by an array of vaccines can each individually mediate protection.

8. Patterns of Host Response

There is a wide spectrum of clinical syndromes associated with infection by *S.* Typhi.[1,2,31,56,153] Many persons exposed to small numbers of bacteria will be asymptomatic and probably will show no clinical signs of infection.[45] A small percentage of infected individuals develop bacteremia but are symptoms free.[154] Perhaps it is from this population that many carriers have developed, as many carriers do not recall having had the disease. The majority of patients with clinically overt disease have a relatively uniform course. A proportion of patients develop severe or complicated disease. In part, the duration of untreated disease prior to initiation of therapy influences the severity of the clinical disease. The curious, severe, clinical form of typhoid fever that was in the past commonly encountered in Jakarta, Indonesia has already been described,[119] as has the atypical generally mild presentation of *S.* Typhi infection observed in infants and toddlers.[31,56]

8.1. Clinical Features

The major signs and symptoms of typhoid fever are relatively nonspecific.[2] There is fever and an associated

moderately severe generalized headache. The temperature increases in a stepwise fashion over the course of 2–3 days, reaching a peak of 103–104°F. During this time, the patient also complains of abdominal pain and may either have diarrhea or (in adults and older children) be constipated. Examination of the abdomen will reveal palpation tenderness in the lower quadrants. Often, one can palpate dilated loops of bowel and sense the displacement of air and fluid under the probing fingers. This indicates that an ileus is present, a probable consequence of the inflammatory process occurring in the lamina propria and Peyer's patches. Late in the first week, careful inspection of the skin may reveal a few rose spots. These are slightly raised erythematous, nontender lesions found in light-skinned persons in the anterior chest or abdominal areas. They initially blanch with pressure but may gradually become fixed as small cutaneous hemorrhages. These lesions are caused by *S.* Typhi organisms infiltrating the endothelial cells of skin capillaries and inducing a perivascular infiltrate and subsequent leaking of blood. Culturing a skin snip of fresh rose spots will yield the organism.[59]

The course of the disease in untreated patients is characterized by a continuous fever with a relative bradycardia in adults. The continuous fever will persist for 2 weeks or longer if no antibiotic is given, and defervescence occurs slowly over the ensuing 2–3 weeks. Such patients have a prolonged convalescence (3–4 months) because of the severe negative nitrogen balance that is a consequence of the prolonged infection. Patients who are receiving an appropriate antibiotic will respond with a gradual reduction in the fever over a 3- to 4-day period.

Complications are the significant prognostic indicators.[1,2] Gastrointestinal hemorrhage or perforation is responsible for many of the fatalities with this disease. Both these events occur after about 2 weeks of untreated disease. The signs and symptoms of a perforated loop of intestine present as an acute abdominal crisis.

8.2. Diagnosis

The diagnosis should be suspected in any patient with fever, abdominal pain, constipation, and headache. A prior history of travel to an endemic area will be an alerting epidemiological fact that should be followed up by appropriate blood and stool cultures. Because of the long incubation (which can be 2 weeks or longer), a thorough epidemiological history should be taken, since the patient may be the index case of a local outbreak. It is important to notify local health authorities promptly.

Typhoid bacilli appear in the blood with the onset of fever, and in untreated patients bacteremia remains for about 2 weeks before abating. The number of organisms in the blood is low, usually less than 10/ml cultured blood. The bacteriological diagnosis is confirmed by positive blood cultures in only about 50–75% of patients. Variables such as prior antibiotic therapy, the volume of blood cultured (the larger the volume, the better), the type of broth used (it should contain an anticomplementary agent), and the number of days that cultures are incubated, all impact on whether a patient with typhoid fever will have a positive blood culture. Diagnosis is confirmed in the remaining patients by bone marrow, bile-stained duodenal string, stool, or rose spot cultures. Stool cultures become positive shortly after ingestion of the organism and may become negative with onset of disease, but may again yield the organism by the end of the first week of disease. Without therapy, stools may readily remain positive for *S.* Typhi for as long as 4 months; beyond this period, continued presence indicates a greater than 90% chance of being a permanent carrier. Antibiotic treatment may cause temporary suppression of the isolation of typhoid bacilli from stools during the therapy.

Antibodies to O and H antigens are already present during the first week of illness.[43] In some of the volunteers with induced disease during studies carried out in the 1960s and early 1970s, the baseline titers prior to challenge with virulent *S.* Typhi were undetectable or low, e.g., 1:20 or 1:40 for O antibodies, yet no rise in titer occurred during the entire time of the prolonged follow-up.[45] These men also had bacteremia and positive stool cultures, an obvious source of antigens.

The differential diagnosis will include other infectious agents that may cause an enteric-fever-like syndrome such as tularemia and other salmonellae. Brucellosis, infectious mononucleosis, dengue fever, and infectious hepatitis may initially be confused because of similar symptoms associated with these febrile illnesses. Leptospirosis may occur with significant abdominal findings and mimic typhoid.

9. Control and Prevention

9.1. General Concepts

Control of typhoid fever requires the identification and tracking of carriers, control of their occupational activities, and, most important, provision of safe water supplies and human waste removal systems. All patients with the disease must be reported to the local health department. Patients should be nursed using enteric precautions. Linen soiled by feces should be handled with disposable gloves and hands should be scrupulously washed following contact with the patient.

Carriers, when discovered, are frequently elderly females. Since the current highly effective treatment regimen

(for susceptible strains) consists of up to 4 weeks of oral ciprofloxacin or norfloxacin,[155,156] care and encouragement must be provided to these elderly individuals during the therapy. If adverse reactions preclude administration of the full course, it is heartening to recognize that even short courses of 10–14 days have succeeded in eradicating the chronic carrier state in certain individuals.[155]

The stringency with which local health departments maintain surveillance of chronic carriers varies greatly. In some areas, these patients are required to submit stool specimens once or twice a year to confirm the continued presence of *S.* Typhi; in other areas, only a listing is maintained. Carriers are prevented from working in any position that could result in their contaminating food or drinking water. This type of control is lacking in most endemic areas, and hence typhoid fever can remain as a common disease.

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis

There is no evidence to support the usefulness of prophylactic antibiotic treatment in preventing disease. To the contrary, data exist that suggest that administration of antibiotics following ingestion of typhoid bacilli but before the onset of clinical illness would not be effective. In tissue cultures, typhoid bacilli persist in the intracellular environment for at least 21 days, despite adequate concentrations of antibiotics in the solution bathing the cells. In the volunteer challenge studies carried out by Hornick et al.[45] four men were begun on chloramphenicol treatment 24 hours after ingesting the 90% infective dose (ID_{90}) of *S.* Typhi. In the two who took the antibiotic for 6 days, the incubation period was delayed by that same period compared to controls. In two men who were maintained on chloramphenicol for 28 days, no disease developed, but there were signs of infection such as positive stool cultures and antibody titer rises.

There is no justification for employing prophylactic antibiotics to prevent typhoid fever. The variable attack rate after known exposure makes it impractical to attempt prophylaxis. If a known common source exposure has occurred, it is much more prudent to maintain surveillance of the potential patients and to treat at the first signs of illness, after obtaining blood and stool cultures. Under these circumstances, treatment with effective antibiotics will reduce the duration and severity of the disease and will prevent complications by limiting the progression of the disease.

9.3. Immunization

The venerable inactivated whole cell parenteral vaccines, including heat-inactivated, phenol-preserved vaccine and acetone-inactivated vaccine, conferred moderate levels of protection for up to 7 years.[18–21,157] However, they were distinctly unsatisfactory vaccines because they caused adverse reactions at such high frequency. In controlled trials, approximately 25% of recipients of the inactivated whole cell vaccines developed fever and systemic reactions and about 15% had to miss work or school.[20,23] For this reason, these vaccines are no longer being manufactured and have been replaced by two modern licensed vaccines: live oral vaccine strain Ty21a (Vivotif) and parenteral purified Vi capsular polysaccharide vaccine (TyphiViM).[3] Both these vaccines confer comparable protection and are well tolerated.

9.3.1. Ty21a. Ty21a, an attenuated strain of *S.* Typhi that is safe and protective as a live oral vaccine, was developed in the early 1970s by chemical mutagenesis of a pathogenic *S.* Typhi strain Ty2.[158] The characteristic mutations in this strain include an inactivation of the *ga*lE gene that encodes an enzyme involved in LPS synthesis and the inability to express Vi polysaccharide. However, approximately two dozen additional mutations are also present in Ty21a. Although Ty21a has proven to be remarkably well tolerated in placebo-controlled clinical trials,[3] it is not clear precisely what mutations in fact are responsible for the stable, impressive attenuation of this vaccine.

Ty21a provides significant protection without causing adverse reactions. Results of multiple double-blind, placebo-controlled studies that utilized active surveillance methods to assess the reactogenicity of Ty21a in adults and children showed that adverse reactions were not observed significantly more often in the vaccine recipients than the placebo group for any symptom or sign. In large-scale efficacy field trials with Ty21a, involving approximately 465,000 schoolchildren in Chile and 32,000 in Egypt, and approximately 20,000 subjects from 3 years of age to adulthood in Indonesia, passive surveillance failed to identify vaccine-attributable adverse reactions or other safety issues.[47–49,52,157,159]

Controlled field trials of Ty21a emphasize that the formulation of the vaccine, the number of doses administered, and the spacing of the doses markedly influence the level of protection that can be achieved.[47–49,52,113,160] Two formulations, including enteric-coated capsules and a "liquid" formulation in which lyophilized vaccine is reconstituted along with buffer powder into a vaccine cocktail, are licensed; however, in recent years only the enteric-coated capsule formulation has been manufactured. Based on a field trial in Chile that demonstrated that three doses of Ty21a in enteric-coated capsules given on an every other day schedule for three dosings conferred 67% efficacy over 3 years of follow-up and 62% protection over 7 years of follow-up,[47,114] this formulation and schedule are used throughout the world except for

Table 5. A Comparison of the Efficacy of Two, Three, or Four Doses of Ty21a Vaccine in Enteric-Coated Capsule Formulation. Results of a Large-Scale, Randomized, Field Trial in Area Sur and Area Central of the Metropolitan Region of Santiago, Chile[a]

Surveillance from 11/84 to 10/87	Two doses	Three doses	Four doses
No. of vaccines	66,615	64,783	58,421
Cases of bacteriologically confirmed typhoid fever	123	104	56
Incidence/10^5	184.6[b]	160.5[c]	95.8[d]
95% confidence interval	152–271	130–191	71–121

[a]Data from Ferreccio et al.[113]
b versus d: $p = 0.00004$; c versus c: $p = 0.002$; a versus b: $p = 0.32$.

North America. The United States and Canada recommend a four-dose regimen with an interval of 48 hours between doses. This was based on results of a large-scale, randomized comparative trial carried out in Santiago, Chile wherein four doses of Ty21a in enteric-coated capsules given within 8 days were shown to be significantly more protective than two or three doses (Table 5).[113]

In the mid-1980s, the Swiss Serum and Vaccine Institute (currently BernaBiotech, a Crucell Company) succeeded in preparing a "liquid suspension" formulation of Ty21a for large-scale field trials that was amenable to large-scale manufacture. The new formulation consisted of two packets, one containing a dose of lyophilized vaccine and the other containing buffer. Contents of the two packets are mixed in a cup containing 100 ml of water and the suspension is then ingested by the subject to be vaccinated. Randomized placebo-controlled field trial were carried out in Santiago, Chile[48] and Plaju, Indonesia[52] to compare directly this new liquid formulation of Ty21a (that somewhat resembles what was used in the Alexandria, Egypt field trial[160]) with the enteric-coated capsule formulation. In both trials the vaccine administered as a liquid suspension was superior to the vaccine in enteric-coated capsules. In the Santiago trial over 3 years of follow-up the liquid suspension formulation conferred 77% efficacy [95% confidence interval (CI), 60–87%], whereas in Indonesia the liquid formulation conferred 53% efficacy (95% CI, 35–66%) Over two additional years of follow-up in the Santiago trial (total 5 years), the liquid formulation conferred 78% efficacy. Ty21a given as a liquid suspension protected young children as well as older children. In previous trials with enteric-coated vaccine, young children were not as well protected as older children.[47]

9.3.2. Vi Polysaccharide Vaccine. In the 1970s and early 1980s, methods were developed to purify Vi capsular polysaccharide so that it was 99.8% free of contaminating LPS and was not denatured.[50,51,81,161,162] This was an important breakthrough because as little as 5% impurity with LPS can result in systemic adverse reactions in a few percent of recipients.[162] In contrast, when Vi vaccine is highly purified, it is well tolerated and febrile reactions are observed in only 1–2% of subjects. In clinical trials, well-tolerated 25 and 50 μg single parenteral doses of purified Vi stimulated rises of serum Vi antibodies in the vast majority of vaccinated adults and school-age children.[50,51,162] Administration of subsequent parenteral doses did not achieve boosts in antibody;[163] thus, the Vi polysaccharide functions like a T-cell-independent antigen. Nevertheless, the antibodies elicited by a single dose persisted for at least 3 years.[164] Passive surveillance carried out during field trials showed the Vi vaccine to be as well tolerated as the licensed (meningococcal and pneumococcal) polysaccharide vaccines that served as the control preparations in these trials.[50,51]

Two randomized, controlled, double-blind field trials were carried out in Nepal and South Africa to assess the efficacy of a single 25 μg dose of nondenatured purified Vi vaccine. Over 17 months of surveillance in Nepal, the vaccine conferred 72% vaccine efficacy.[51] In South Africa, the vaccine provided 64% protection over 21 months of follow-up and 55% protection over 3 years.[50] The Nepal trial included all ages from pre-school to adulthood, whereas the South African trial was performed in schoolchildren. A third controlled field trial was carried out in subjects 3–50 years of age in Guangxi, China that evaluated the protective efficacy of a single 30 μg dose of a Vi polysaccharide vaccine manufactured in China.[53] The vaccine conferred 69% efficacy (95% CI = 28%, 87%) over 19 months of follow-up.

Beyond its safety and efficacy in school-age children and adults, an advantage of the Vi vaccine is that it provides a moderate level of protection after just a single dose. On the other hand, there are no robust data to suggest that the efficacy of Vi persists beyond 3 years; efficacy was 55% at 3 years in the South African trial. This concern over the relatively short-lived duration of protection of Vi has been heightened following an epidemiologic investigation of an outbreak of typhoid fever that occurred among Vi-vaccinated French soldiers deployed to Ivory Coast.[125] Prior to the outbreak the standard operating procedure had been to immunize French soldiers with Vi vaccine every 5 years. The outbreak investigation revealed that receipt of Vi more than 3 years earlier was associated with a significantly increased risk of developing typhoid fever during the outbreak.[125] Table 6 summarizes the salient characteristics of Ty21a and Vi vaccines.

9.3.3. New Generation Typhoid Vaccines. New strains of *S.* Typhi that have been genetically engineered to contain precise attenuating mutations have been successfully tested in Phase 1 and 2 clinical trials and shown to be

Table 6. A Comparison of the Characteristics of Live Oral Vaccine Ty21a and Parenteral Vi Polysaccharide Vaccine

	Ty21a	Vi
Type of vaccine	Live	Subunit
Route of administration	Oral	Parenteral
Immunization schedule	3 or 4 doses (given every other day)	1 dose
Cold chain required	Yes	Yes
Well tolerated	Yes	Yes
Efficacy	60–96%	64–72%
Duration of efficacy	7 years	3 years
Evidence of a herd immunity effect	Yes	Yes
Interferes with use of serum Vi antibody as a screening test to detect chronic typhoid carriers	No	Yes

well tolerated and immunogenic after ingestion of just a single oral dose. The live oral vaccine candidates include strains M01ZH09,[165–167] Ty800,[168] CVD 908-*htrA*[169,170] and CVD 909.[171]

Booster doses of purified Vi do not raise antibody titers over those elicited by a single dose of vaccine; i.e., immunologic memory does not appear to occur. To remedy this, Vi polysaccharide has been conjugated to a carrier protein (recombinant exotoxin A of *Pseudomonas aeruginosa*) to increase its immunogenicity by conferring T-cell-dependent properties upon the antigen, including the induction of immunologic memory. In children and adults in endemic areas booster doses of Vi conjugate vaccine clearly increase the titers of antibody over those elicited by a priming dose.[54,55,172] A randomized, controlled field trial of Vi conjugate in children immunized at ages 2–4 years in the Mekong Delta of Vietnam demonstrated 91.5% (77.1–96.6%) vaccine efficacy over 27 months of active surveillance[54] and 82% efficacy during an additional 19 months of follow-up that utilized a passive surveillance system.[55]

10. Unresolved Problems

Areas ripe for research include (1) studies of the role of cytokines and immunopathology in the pathogenesis of typhoid fever; (2) studies of the role and mechanisms of cell-mediated immunity in protection against typhoid fever and the degree to which these are stimulated by live oral vaccine strains[173]; (3) studies of the genetic basis of human susceptibility to clinical typhoid fever[122]; (4) studies to ascertain why infants and toddlers typically manifest mild clinical responses in the presence of bacteremic infection by wild-type *S.* Typhi[56]; (5) the development of practical, inexpensive, rapid assays to make a microbiological confirmation of typhoid fever with a high degree of sensitivity and specificity;[174] and (6) investigations to determine why *S.* Typhi does not readily carry R factor plasmids other than those of incompatibility group H1; (7) further studies of the epidemiology of typhoid and paratyphoid fever in infants, toddlers and pre-schoolchildren that utilize both active and passive surveillance in different sites within the same general population. Finally, should the new generation of recombinant live oral vaccines and parenteral conjugate vaccines realize their potential of being well-tolerated, highly immunogenic and protective public health tools, attention will have to be given to the possibility of worldwide control of typhoid fever. In typhoid-endemic areas, such an undertaking would involve enhanced provision of water supplies and means for human waste disposal, mass vaccination of schoolchildren with the new generation of vaccines, and identification and treatment of epidemiologically relevant carriers.

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CHAPTER 44

Yersinia enterocolitica Infections

Ann M. Schmitz and Robert V. Tauxe

1. Introduction

Three bacteria of the genus *Yersinia* are recognized human pathogens. *Yersinia pestis* is the cause of plague, a devastating epidemic disease transmitted from rodent reservoirs to humans by the bite of the flea. *Y. pestis* evolved from *Yersinia pseudotuberculosis*, a cause of epizootic disease in animals and mesenteric lymphadenitis in humans, that can be transmitted through contaminated food.[1] *Yersinia enterocolitica*, recognized as a cause of human illness around the world, also causes intestinal infections and is transmitted from animal reservoirs through contaminated food. The various strains of *Y. enterocolitica* form a heterogenous group of bacteria that includes both well-established pathogens and non-pathogenic environmental strains that are ubiquitous in terrestrial and freshwater ecosystems. Only a few phenotypic variants have been conclusively associated with human or animal disease.[2–4] The principal pathogens for humans are members of a few serogroups (O:3, O:9, O:8, O:5,27). Other strains have been associated with important animal diseases, but the strains that cause human illness are carried asymptomatically in their animal reservoirs and differ from those strains that cause disease in animals.

Illness caused by *Y. enterocolitica* and *Y. pseudotuberculosis* is referred to as yersiniosis. *Y. enterocolitica* is the etiologic agent of a range of clinical entities in humans, although acute enterocolitis is the most frequent manifestation. The bacterium also causes postinfectious sequelae, most notably reactive arthritis. *Yersinia pseudotuberculosis* causes enteritis and pseudoappendicitis, and may also be transmitted through contaminated foods, and is only common in northern Eurasia.[5] The present discussion will focus on *Y. enterocolitica* and its role in a variety of clinical syndromes afflicting humans.

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2. Historical Background

A description of the organism now known as *Y. enterocolitica* was first published in 1939, by researchers at the New York State public health laboratory, who reported "an unidentified microorganism resembling *Bacterium lignieri* and *Pasteurella pseudotuberculosis* and pathogenic for man", which they subsequently named *Bacterium enterocoliticum*.[6] Similar organisms were reported by various researchers in the following 20 years and given various names, including *Pasteurella X, Y. pseudotuberculosis* type B, *B. enterocolitica*, and Les Germes X.

Following widespread epizootics among chinchillas and hares and after the establishment of a causative relationship with lymphadenitis in man, and in 1964, Fredericksen proposed the species name *Y. enterocolitica* for this taxon.[7] The species *Y. pseudotuberculosis* was distinguished from *Y. enterocolitica* at this time and has since emerged as an important cause of infection in northern Russia and Finland, where it causes illness in hares and deer, as well as human outbreaks that have been associated with consumption of fresh produce.[5,8]

In the 1970s, the organism was recognized worldwide, as improved methods of stool culture facilitated isolation of the organism. While better diagnosis as important, it also appears likely that a global dissemination of pathogenic strains occurred at that time.[9] The recognized incidence of the infection in Belgium and in some other countries began to increase dramatically.[10] Coincident with this heightened

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awareness, it became evident that the original biochemical criteria proposed for the species *Y. enterocolitica* encompassed a heterogeneous group of bacteria. Many "*Y. enterocolitica*-like organisms" were described that differed substantially in their biochemical reactions, and several biotyping and serotyping schemes were proposed.[11–13] DNA hybridization techniques finally divided this diverse group of microbes into eight species with distinct biochemical profiles: *Y. enterocolitica, Y. frederiksenii, Y. kristensenii, Y. intermedia, Y. aldovae, Y. rohdei, Y. mollaretii*, and *Y. bercovieri*.[14–16] "*Y. ruckeri*", an economically important pathogen of farmed fish was proposed as a species in 1978.[17,18]

3. Methodology

3.1. Sources of Mortality Data

In developed countries, where most reported yersiniosis cases occur, infection with *Y. enterocolitica* is rarely fatal. Infection most commonly results in enterocolitis. As for many infections, the mortality associated with *Y. enterocolitica* can be difficult to determine, since mortality data are often not collected in pathogen-specific surveillance, and death certificates tend to underreport many pathogen-specific conditions. Pathogen-specific case-fatality has been reported in the active surveillance system, Foodborne Diseases Active Surveillance Network (FoodNet), in the United States,[19] and was included in an overall burden of illness estimate.[20] In a registry-based study linking diagnosis with vital statistics in Denmark, the excess mortality following diagnosis of *Y. enterocolitica* infection could be compared with the mortality in the general population.[21] Syndrome-specific fatality rates for gastroenteritis, mesenteric adenitis, and terminal ileitis are not known; the few fatal cases reported for these clinical syndromes have been attributed to severe complications such as intestinal ulceration and peritonitis. Extraintestinal manifestations of intestinal *Y. enterocolitica* infection are usually self-limited. Clinical disease due to septicemia, most often reported in children or adults compromised by severe anemia, hemochromatosis, cirrhosis, malignancy, malnutrition, or immunosuppressive therapy, is associated with higher rates of mortality. In one nosocomial outbreak, a case–fatality ratio of approximately 50% was reported in 13 patients with disseminated *Y. enterocolitica* infection and a universal mortality in those with underlying hemochromatosis.[22] The complications resulting from disseminated infection, which include suppurative hepatic, renal, and splenic lesions, osteomyelitis, wound infection, or meningitis, and from transfusion-associated yersiniosis, also carry a higher risk of death.

3.2. Sources of Morbidity Data

Many countries maintain surveillance systems to detect infections and estimate burden of illness attributable to specific pathogens, though reporting requirements may vary substantially.[23] In the United States, *Y. enterocolitica* is not nationally reportable and is reportable by law in only 26 states.[24] In 1996 the Foodborne Diseases Active Surveillance Network (FoodNet) began active surveillance for infection by *Y. enterocolitica*, and by other pathogens often transmitted by foods, and now provides annual incidence data.[25] In other countries, such as Sweden, Finland, Ireland and New Zealand, yersiniosis cases are nationally notifiable. Since many clinical laboratories in most countries do not routinely screen diarrheal stool specimens for *Y. enterocolitica*, it is likely to be underdiagnosed and underrecognized by clinicians, and underreported to surveillance. Lack of universal application of laboratory techniques that favor its isolation, as well as incomplete reporting of *Y. enterocolitica* isolates to public health agencies, also limits the completeness of laboratory-based surveillance. Outbreak investigations can provide some additional morbidity data.

3.3. Surveys and Investigations

Surveys of the relative frequency of *Y. enterocolitica* in populations with diarrhea have justified its inclusion as a bacterial enteric pathogen of importance in some parts of the world (Section 5.1). Studies in England and Australia have estimated the pathogen-specific burden of foodborne disease including that caused by *Y. enterocolitica.*[26,27] Serological surveys have been conducted in several European countries (reviewed in[28]). However, direct comparison of the results is difficult, since different subpopulations were studied and since a variety of serological assays and antigens were used.

3.4. Laboratory Diagnosis

3.4.1. Isolation and Identification of the Organism. *Y. enterocolitica* belongs to the family *Enterobacteriaceae*.[29] It is a gram-negative, oxidase-negative, catalase-positive, nitrate-reductase-positive, facultative anaerobic rod that is typically 0.5–0.8 × 1–3 μm in size. Coccobacillary forms may be seen. It does not form a capsule or spores. *Y. enterocolitica* is nonmotile at 35–37°C but motile at 25–27°C, with relatively few, peritrichous flagellae. Some strains of serogroup O:3, however, are nonmotile at both temperatures. In addition, the bacterium is urease-positive, ferments mannitol, and produces acid

but not gas from glucose. *Y. enterocolitica* differs from most members of the family *Enterobacteriaceae* by virtue of its slower growth in general and its ability to grow at refrigerator temperatures.

Bacteriological isolation of the organism is by far the method of choice for diagnosing *Y. enterocolitica* infection. The isolation and identification of the bacterium from normally sterile sites like blood are not difficult using standard bacteriological procedures. Recovery of the organism from stool, on the other hand, is hampered by the lack of distinctive colonial morphology as well as by the organism's slow growth and the resultant overgrowth of normal fecal flora. Several selective media have been developed to enhance the recovery of *Y. enterocolitica* from stool specimens. While routine enteric media can be used in experienced hands, isolation is more easily accomplished on cefsulodin–irgasan–novobiocin (CIN) agar, a highly selective medium used to recover *Y. enterocolitica* from stools. On this agar, *Y. enterocolitica* appears as 0.5- to 1.0-mm diameter colonies with a dark red "bull's eye" and transparent border. CIN agar permits the growth of all pathogenic serogroups.[30] Two selective media, *Salmonella–Shigella*–deoxycholate–calcium (SSDC) agar and irgasan–ticarcillin–potassium chlorate (ITC) enrichment broth, have been specially designed for isolation of O:3, the most common serogroup.

The use of a cold enrichment step at 4°C can facilitate recovery of *Y. enterocolitica* when the bacterial density is low, such as in the convalescent phase of an infection. However, this procedure may require incubation for weeks and is likely to lead to isolation of non-pathogenic strains, making it of little clinical use.[31] Isolates of *Y. enterocolitica* identified in this fashion must be further characterized before diagnostic conclusions are drawn.

Y. enterocolitica is quite heterogeneous with respect to phenotypic, genotypic, and ecological properties. The phenotypic heterogeneity has enabled the development of several schemes for subdivision of the bacterium. Serogrouping according to O antigens and differentiation of biotypes on the basis of biochemical properties have been the most useful typing methods. *Y. enterocolitica* has been divided into approximately 60 serogroups using O antigens.[13] Several O antigen groups may be further subdivided by H antigens. However, the use of H-antigen typing has been hampered by its variable expression and limited availability. The biotyping scheme proposed by Wauters has been widely used; a revision was published in 1987.[32] In addition to serogrouping and biotyping, a number of phenotypic and genotypic methods have been used to subdivide *Y. enterocolitica*. The discriminatory power of the genotypic methods may be higher for serogroup O:8 than for O:3, O:9, and O:5,27.[33] Molecular subtyping as part of public health surveillance to improve the detection of diffuse outbreaks has not yet been implemented in a routine way for *Y. enterocolitica*, as it has been for *Escherichia coli* O157 and *Salmonella* in the US PulseNet system, though it is likely to be in the future.

In clinical and research laboratories, subtyping is important because *Y. enterocolitica* strains vary in their ability to cause disease, and many strains have no established pathogenic significance. Surveys of the environment or of foods conducted without subtyping may identify many *Y. enterocolitica* strains that are of no public health significance. Fortunately, the great majority of the strains that cause illness in humans or animals belong to only a few serogroups and biovars.[2] Somewhat simplified, *Y. enterocolitica* may be divided into three groups according to clinical significance: human pathogens, animal pathogens, and environmental strains, each with different serogroups. The most important human pathogens are O:3 biovar 4; O:5,27 biovar 2; O:8 biovar 1B; and O:9 biovar 2, though other serogroups may occasionally cause infection.

The strains that are pathogenic for animals include O:1 biovar 3, responsible for widespread outbreaks in chinchillas; and O:2 biovar 5, associated with disease in hares, sheep, and goats.[34] The environmental strains include a spectrum of phenotypic variants with a variety of other serogroups; most belong to biovar 1A. These bacteria usually lack human clinical significance. Such environmental strains have occasionally been involved in human infection, mainly in immunocompromised patients and patients with underlying conditions.

Y. enterocolitica may be differentiated from other species of the genus by means of several distinct biochemical reactions, and other phenotypic differences. The "*Y. enterocolitica*-like organisms" include *Y. frederiksenii, Y. kristensenii, Y. intermedia, Y. aldovae, Y. rohdei, Y. mollaretii*, and *Y. bercovieri*.[29] Such species are frequently encountered in terrestrial and freshwater ecosystems. Like the environmental *Y. enterocolitica* strains, these species have not been associated with human or animal disease, with rare and atypical exceptions. *Y. ruckeri*, an important cause of illness in farmed trout, is not linked to human illness.[18]

3.4.2. Serological Diagnostic Methods. Serodiagnosis has been developed for research purposes, but is not often used for routine diagnosis.[35] Infection with *Y. enterocolitica* elicits an immunologic response that can be measured by a variety of techniques, including tube agglutination, indirect hemagglutination, enzyme-linked immunosorbent assay, and indirect immunoflurorescent-antibody assay. [36–38]

Agglutinating antibodies appear soon after the onset of illness and persist for 2–6 months. Patients with systemic infection produce a higher antibody titer than do persons with disease restricted to the gastrointestinal tract.[35]

Cross-reactions complicate the serological diagnosis of *Y. enterocolitica* infection. Some of these are with other bacteria. For example, *Y. enterocolitica* serogroup O:9 shares cross-reacting antigens with *Brucella.*[29] Antibodies to thyroid hormone receptors, which are elevated in autoimmune hyperthyroiditis (Graves disease), have been described that also have a cross-reacting affinity to *Y. enterocolitica* antigens, though the bacteria play no role in thyroiditis.[39,40] The detection of antibodies by immunoblots to certain plasmid-encoded proteins, the *Yersinia* outer membrane proteins (Yops), has been suggested as a highly specific method to demonstrate previous *Y. enterocolitica* infection.[41,42] Demonstration of specific circulating IgA to the Yops is indicative of recent or persistent infection and is strongly correlated with the presence of virulent *Y. enterocolitica* in the intestinal lymphatic tissue of patients with reactive arthritis.[43]

4. Biological Characteristics of the Organism

Y. enterocolitica is psychrotrophic, which means it can multiply at temperatures approaching 0°C, and grow in properly refrigerated foods.[44] This provides *Y. enterocolitica* with a competitive advantage over many other bacteria for propagation in environmental reservoirs. The growth of *Y. enterocolitica* on cooked meats and in milk at low temperatures is well documented. However, the presence of other psychrotrophic organisms in food may inhibit growth of *Y. enterocolitica*.[45,46] *Y. enterocolitica* can survive in frozen foods for long periods.[44] Most virulent strains behave differently depending on temperature. At room temperature (25–27°C), they express flagella and so are motile and express few virulence factors; while at 37°C, most strains are nonmotile, but express an array of virulence factors.[2,47] This temperature dependence of biologic characteristics may be important for survival of the organism outside of the host and for pathogenesis in the host.

Y. enterocolitica is relatively iron dependent. Unlike most other aerobic bacteria, most strains of *Y. enterocolitica* do not produce iron-binding compounds, or siderophores, and instead depend on siderophores produced by other bacteria to capture the iron needed for growth. They are also able to use hemin as a source of iron.[48] Particularly severe infections can occur in patients who already have conditions of iron overload, especially during treatment with iron chelators that mobilize body iron, and may make it more easily available.[49] *Y. enterocolitica* are also relatively calcium dependent, and most virulent strains require calcium-supplemented media to grow at 37°C. It is thus not a great surprise that outbreaks of yersiniosis are often traced to vehicles that are rich in calcium and iron and that are held refrigerated and consumed cold.

The virulence of human pathogenic strains of *Y. enterocolitica* has been intensively studied and depends on a variety of molecules and mechanisms. Several of these are mediated by the virulence plasmid of approximately 70 MD, known as pYV.[50] The presence of the plasmid is tightly correlated with calcium-dependent growth at 37°C. Similar plasmids are also present in *Y. pseudotuberculosis* and *Y. pestis.* Although many virulence determinants are encoded on the chromosome, the presence of the plasmid is an important marker for virulence. The same plasmid also carries a gene for arsenic resistance, and it has been suggested that the widespread use of arsenicals in the past to treat swine dysentery and other infections of pigs may have selected for the presence of the plasmid.[51]

Production of urease occurs at 25–27°C. The action of this enzyme releases ammonia from urea that is present in the mucous layer of the stomach, buffering local gastric acidity, and providing relative acid resistance.[52] Adherence and invasion of the mucosal cells occurs via invasin and other surface proteins that are chromosomally encoded.[53] The organism produces several additional proteins that enable it to escape host defense mechanisms, including phagocytosis and the bactericidal action of serum. Two such proteins, Ail (attachment invasion locus) and YadA (*Yersinia* adhesin A), are adhesins that also confer resistance to complement-mediated opsonization. The most complex of these virulence proteins are the plasmid-mediated*Yersinia* outer membrane proteins (Yops).[54] The Yops are not simple outer membrane proteins, but an extraordinary array of effectors that are produced in the bacteria, then secreted and inserted into host cells via a type III secretion system.[55] The injector apparatus is assembled and armed on the surface of the bacteria at 37°C, and the Yops are injected on contact with the target cell. The injected Yops have an array of effects on the cells of the mammalian host. They rapidly paralyze phagocytes, block secretion of recruitment molecules such as TNF-alpha and IL-8, and appear to inhibit activation of macrophages.[55,56] The cumulative effect is that inflammation is suppressed and phagocytosis evaded. In addition, a few strains have genes for an iron-binding siderophore known as yersiniabactin, which can efficiently bind iron in iron-deprived sites, permitting continued rapid growth.[57] Similar virulence mechanisms have been described in *Yersinia pseudotuberculosis*.

While virulence factors for attachment and invasion explain invasion and systemic infection, they do not by themselves account for diarrheal illness. A better candidate for that characteristic is a specific *Yersinia* heat-stable

enterotoxin, Yst, which is similar to the heat-stable enterotoxin of enterotoxigenic *E. coli.* This toxin is produced under a specific combination of temperature, pH, and osmolality resembling that of the mammalian ileum.[58,59] It is found almost exclusively and universally in pathogenic strains of *Y. enterocolitica.*[60]

Not all strains possess all these mechanisms. The so-called high pathogenicity island that codes for yersiniabactin and perhaps other virulence factors is present only in *Y. pseudotuberculosis* and biotype 1B of *Y. enterocolitica.*[57] Although in general, *Y. enterocolitica* biotype 1A has been regarded as avirulent, some strains have been described that appear to be enteropathogenic, though they lack pYV and other known virulence determinants.[61,62] It is likely that more virulence determinants remain to be identified.

A number of assays have been used to differentiate pathogenic and non-pathogenic variants. Most test for the presence of the pYV plasmid, using such markers as autoagglutination at 37°C, calcium-dependent growth restriction at 37°C, resistance to the bactericidal activity of normal human serum, binding of Congo red dye, ability to invade various cell cultures, or by direct DNA sequence probing.[44,6364] Autoagglutination and calcium-dependent growth have been the most widely used in vitro tests, though no single property appears to be an absolutely reliable indicator of the pathogenic potential. The striking correlation between potential pathogenicity and lack of pyrazinamidase activity, independent of the occurrence of the virulence plasmid, makes this a useful test, which is included in the revised Wauters biotyping scheme.[32] One practical approach combines pyrazinamidase, calcium-dependence, Congo red dye uptake, and phenotypic assays to screen for pathogenic strains.[65]

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

Y. enterocolitica infections were recognized in the 1970s when global awareness increased and a widespread dissemination may have occurred with real subsequent increases in incidence.[9,66] In Belgium, Finland, Germany, New Zealand and Sweden, *Y. enterocolitica* surpassed *Shigella* to rival *Salmonella* as a cause of acute bacterial gastroenteritis. Recently reported incidence varies substantially from country to country, from <0.1 per 100,000 in France and Portugal to 13.6 per 100,000 in Lithuania (Table 1).

Several studies have estimated the overall burden of illness due to yersiniosis, adjusting for likely underreporting. In an analysis based on FoodNet data, the reported incidence of positive cultures in the time span 1996–1999 was 0.8/100,000, but after adjusting for the proportion of laboratories that do not routinely seek the organisms that incidence was estimated to be 5/100,000 among those ill enough to seek care and be cultured.[67] Broader approaches that estimate the numbers of all illnesses, rather than just those being cultured, arrive at substantially higher estimates. It was estimated that in 1997 in the United States, *Y. enterocolitica* caused 96,000 illnesses, 1,228 hospitalizations, and 3 deaths, of which 90% was foodborne in origin, yielding an estimated incidence of 36 per 100,000.[20] Using a similar approach, Australian investigators estimated that in 2000, yersiniosis accounted for 2,200 infections and 34 hospitalizations, of which 75% was foodborne in origin, an estimated incidence of 115 per 100,000.[27] Using data derived from reported foodborne outbreaks, English investigators estimated that the annual burden of yersiniosis in England and Wales, 1996–2000, was 129,000 cases of illness, 619 hospitalizations, and 3 deaths, an estimated annual incidence of 250 per 100,000.[67a]

Table 1. Annual Incidence of *Yersinia* Infections in Selected Countries with Surveillance

Country	Year	Incidence rate per 100,000 population	Reference
Australia	2004	1.3	[79]
Austria	2004	1.4	[80]
Belgium (Flemish Community)	2004	4.8	[80]
Czech Republic	2004	4.9	[80]
Denmark	2004	4.2	[80]
Estonia	2004	1.1	[80]
Finland	2004	13.1	[80]
France	2004	<0.1	[80]
Germany	2004	7.5	[80]
Greece	2004	0.4	[80]
Hungary	2004	0.7	[80]
Ireland	2004	0.1	[80]
Italy	2004	0	[80]
Latvia	2004	1.1	[80]
Lithuania	2004	13.6	[80]
New Zealand	2005	10.9	[72]
Norway	2004	2.2	[80]
Poland	2004	0.2	[80]
Portugal	2004	<0.1	[80]
Slovakia	2004	1.4	[80]
Slovenia	2004	1.9	[80]
Spain*	2004	0.5	[80]
Sweden	2004	9.0	[80]
United Kingdom	2004	0.1	[80]
United States	2005	0.36	[25]

*Only hospitalized cases are reported.

The incidence of illness in a country may change importantly over time. In Belgium, surveillance was started in 1967[68] and documented an important increase in incidence in the 1970s.[10] Some of the early part of the increase may have been due to improved diagnosis and reporting, but much of it appeared to be a real increase. The number of cases reported quadrupled from 305 cases in 1975 to 1,469 in 1986, an incidence of approximately 14.7 per 100,000, and then declined to 7.1 per 100,000 by 1996.[68] As in other European countries, serotype O:3 was the dominant serotype, representing 79% of strains from 1967 to 1996, though this proportion dropped over time. Factors thought to play a role in this increase included changes in the breeding practices of swine, in slaughter methods that include trimmed tonsil meat in ground pork, and traditional food habits in Belgium which include regular consumption of undercooked or raw ground pork.[69] Changes in slaughter practice to reduce contamination of ground pork with tonsil tissues, and an education campaign about the hazards of raw pork may have contributed to the decline. In the United States, the incidence of yersiniosis tracked in FoodNet has decreased by 49% from 1996 to 2005, for reasons that remain to be clarified.[25]

In New Zealand, infections due to *Y. enterocolitica* has increased since the late 1980s.[70] The frequency of isolation of *Y. enterocolitica* from specimens in the Auckland area rose from 0.2% in 1987 to 0.5% in 1989. The most common serogroups seen at that time were O:3 (92%) followed by serogroup O:5/27 (4%) of isolates. In a study conducted on stool samples collected in the Auckland area 1988–1993, *Y. enterocolitica* was identified as the third most common enteric pathogen isolated after *Campylobacter* and *Salmonella*.[71] Serogroup O:3 was most frequently recovered and there was no seasonal pattern. Since the 1990s, the incidence of yersiniosis in New Zealand has been one of the highest in the world and was 10.9 per 100,000 in 2005.[72]

The reported proportion of acute diarrheal illness due to *Y. enterocolitica* has varied depending on geographic location, time, population, and study. In one study conducted at the peak of the Belgian epidemic, the percentage of routine stool cultures yielding *Y. enterocolitica* of serogroups O:3 and O:9 was as high as 2.5%.[73] In an early study in Canada, *Y. enterocolitica* was isolated from 2.8% of the stools submitted at pediatric hospitals.[74] The frequency of yersiniosis was greater than shigellosis (1.1%) and less than salmonellosis (4.4%) and was most commonly associated with serogroup O:3. In a study of pediatric gastroenteritis in the United States, conducted at the highest risk time of year, and in a city and population known to be affected, 76 (1%) of 7,290 stool cultures yielded *Y. enterocolitica.*[75] Studies with lower yield have also been published. In a study in the Netherlands, conducted in 1996–1999 to determine the overall burden of enteric infections, 5 (0.5%) of 857 stools from diarrheic patients yielded *Y. enterocolitica*, but none of the 5 were pathogenic serotypes, and the yield was no different from the cultures of healthy controls.[76] Similarly, an exhaustive study of pediatric gastroenteritis recently conducted in Seattle in the United States found only 2 (0.1%) of 1,626 stools yielded *Y. enterocolitica.*[77]

The yield of diagnostic culture may be higher in specific syndromes. Several studies of patients with the appendicitis-like syndrome have found *Y. enterocolitica* in up to 9% of patients. In Belgium, the isolation rate of *Y. enterocolitica* from appendices removed for clinical appendicitis was 3.8%.[73] In a report of 581 persons who underwent appendectomy for presumed appendicitis over a 1-year period in Sweden, 22 cases (3.8%) had bacteriological evidence of *Y. enterocolitica* infection.[78] Of those found to have regional terminal ileitis at surgery, 80% were infected with *Y. enterocolitica*, as were 13% of those with mesenteric adenitis, and 0.5% of those with acute appendicitis. Although sometimes present in asymptomatic individuals, surveys have found carriage rates of less than 1% in asymptomatic controls.[74]

The case–fatality rate reported for diagnosed cases of *Y. enterocolitica* infection in FoodNet was 0.77% for the period 2000–2004.[19] The crude 30 day excess mortality reported from the Danish registry study was 0.14%.[21] The same investigators reported that the death rate was significantly elevated out to 180 days after diagnosis even after adjusting for underlying illness.

5.2. Epidemic Behavior and Contagiousness

Outbreaks of yersiniosis traced to specific sources are unusual, but illuminating (Table 2). It is curious that in recent years, while both clinical awareness and public health surveillance for yersiniosis have been good and improving in a number of countries, very few outbreaks are reported. For example, in 2004, none were reported in Australia,[79] and only two, affecting a total of eight persons were reported in New Zealand.[72] In 2004 in Europe, a total of 51 outbreaks of yersiniosis were reported, affecting a total of 182 persons. However, for only two outbreaks was a source was reported, one of which was an outbreak of *Yersinia pseudotuberculosis* infections in Finland affecting 58 persons.[80] The remaining outbreaks, affecting a mean of 2.5 persons each, were reported from Austria, Czech Republic, Denmark, Germany and Portugal, and a source (raw ground beef) was identified for only one. While few well-characterized outbreaks of enteritis due to *Y. enterocolitica* have been reported in Europe, several major outbreaks have been described in the

Table 2. Outbreaks of *Y. Enterocolitica* Infections with Identified Route and Vehicle of Transmission

Author	Year published	Location	No. ill	Mode of transmission/vehicle	Serogroup	Reference
Gutman et al.	1973	North Carolina (family)	16	Contact with sick puppies?	O:8	[81]
Toivanen et al.	1973	Finland (hospital)	6	Nosocomial	O:9	[109]
Black et al.	1978	New York (school)	222	Chocolate milk	O:8	[82]
Aber et al.	1982	Pennsylvania (Brownie troop)	16	Bean sprouts (contaminated by water)	—	[90]
Ratnam et al.	1982	Canada (hospital)	9	Nosocomial/ (person-to-person?)	O:5	[105]
Morse et al.	1984	New York (summer camp)	239	Powdered milk or chow mein (contaminated by food handler)	O:8	[84]
Tacket et al.	1984	Tennessee, Arkansas, Mississippi	>172	Pasteurized milk	O:13,18	[83]
Tacket et al.	1985	Washington	44	Tofu (contaminated by water)	O:8	[89]
Maruyama	1987	Japan (school)	1,051	Milk	O:3	[91]
Lee et al.	1990	Georgia	15	Indirect contact with preparation of chitterlings	O:3	[88]
Greenwood et al.	1990	United Kingdom (hospital)	36	Pasteurized milk	O:10, O:6,30	[85]
Ackers et al.	2000	Vermont and New Hampshire	10	Pasteurized milk	O:8	[87]
CDC	2003	Illinois	9	Contact with preparation of chitterlings	O:3	[115]
Jones	2003	Tennessee	12	Contact with preparation of chitterlings	O:3	[114]
Sakai	2005	Japan (nursery school)		Cold mixed salad	O:8	[93]

United States. The first of these was puzzling. In 1972, 16 of 21 individuals from four linked families were affected in rural North Carolina.[81] The outbreak resulted in two exploratory laparotomies and two of the afflicted individuals died, one following surgery. A serogroup O:8 *Y. enterocolitica* was isolated from the spleen of a fatal human case, and eight others had serologic evidence of recent infection. Although the source was not confirmed, five of nine newborn puppies associated with one family had died with diarrheal illness during the week before the first human illness.

Food is the most common vehicle of transmission in outbreaks of *Y. enterocolitica* infection. Several outbreaks involved commercial milk, which likely became contaminated after pasteurization. The first of these occurred in 1976, in upstate New York.[82] Illness occurred in 222 school children and employees, 38 of whom had documented *Y. enterocolitica* serogroup O:8 infection; 36 individuals were hospitalized and 16 appendectomies were performed. Illness was associated with the consumption of chocolate milk purchased in the school cafeteria. *Y. enterocolitica* was subsequently isolated from a carton of chocolate milk. As no defects in pasteurization were identified, it was presumed that contamination occurred afterwards, possibly during introduction of chocolate flavor or vitamins.

In 1982, a multistate outbreak caused by serogroups O:13 and O:18 caused illness in at least 172 persons; 17 individuals underwent appendectomy and 24 cases of extraintestinal disease were documented.[83] Illness was associated with drinking pasteurized milk from a Memphis, Tennessee plant. Samples from the incriminated lots of milk were not available for culture. Again, no flaws in pasteurization were identified. A survey revealed that 8.3% of those who drank the milk became ill; the attack rate was 22.2% in children 0–4 years old and 38.5% in children 5–9 years old. In a third outbreak, 53% of persons at a camp experienced illness caused by *Y. enterocolitica* serogroup O:8, and the investigation showed that illness was associated with consumption of reconstituted powdered milk and/or chow mein.[84] The

organism appeared to have been introduced by an ill food handler during preparation.

In 1985, a cluster of infections with *Y. enterocolitica* serogroups O:10 and O:6,30, biovar 1 was identified in a pediatric ward in Great Britain, and few of the children had diarrhea or abdominal pain; culture of the feces of 34 yielded one or the other serogroups.[85] The same strains were isolated from pasteurized milk from a local dairy, delivered to the hospital. Intensive investigation of the dairy indicated that although there was no defect in the pasteurization itself, the organism could be recovered from freshly pasteurized milk from the bottle filling line, before it entered the bottles, though not from milk from a carton filling line; a nidus of contamination in the bottle filling apparatus was suspected.[86] In 1995, an outbreak of serogroup O:8 caused illness in 10 patients from Vermont and New Hampshire in 1995.[87] Three of these patients were hospitalized and one underwent appendectomy. Consumption of pasteurized milk from a local dairy was significantly associated with disease. *Y. enterocolitica* was isolated from one raw milk sample and from a fecal sample from a dairy pig. As no defects in pasteurization or filling apparatus were identified, it was suspected that the organism could have been transferred in some fashion from the pigs to the clean bottles waiting to be filled.

Pork is the most important source of sporadic cases of yersiniosis and has also been identified as the vehicle of community outbreaks. In the United States, an outbreak of bacteremia and gastroenteritis was recognized in African-American infants in the winter of 1988–1989.[88] The infants were all formula fed, but the formula was of a variety of brands and types, and so was unlikely to be the source of contamination. The ill infants had been cared for by persons who were simultaneously preparing chitterlings, a dish of boiled pork tripe. In the course of preparation, *Yersinia* appears to have been transferred from the raw tripe to the infants on the unwashed hands of the caregiver. *Y. enterocolitica* serogroup O:3 biovar 4 was isolated from 8 of 15 samples of raw pork tripe, as well as from the patients. This outbreak heralded the emergence of this subtype in the United States.

Water may also be a source of contamination of foods. An outbreak of 44 *Y. enterocolitica* infections occurred in Washington between December 1981 and February 1982.[89] Disease was associated with the ingestion of contaminated tofu (soybean curd), and *Y. enterocolitica* serogroup O:8 was isolated from afflicted individuals. Culture of the tofu, as well as of the untreated spring water used in the tofu manufacturing plant, yielded the same serogroup, O:8. A second outbreak involved 16 of 33 (48%) members of a Brownie troop who were infected following the ingestion of bean sprouts that had been grown in refrigerated water from a well that was shown to be contaminated with *Y. enterocolitica*; 15 of the children manifested gastrointestinal symptoms an average of 6 days following exposure and three underwent appendectomy.[90]

In Japan, epidemic *Y. enterocolitica* enteritis has been described in communities and schools, but without epidemiologic or microbiologic implication of specific sources.[91,92] One such outbreak occurred in 1972 in a Japanese junior high school where acute abdominal pain and fever were present in 198 of 1,086 pupils.[92] *Y. enterocolitica* serogroup O:3 was isolated from the fecal specimens of the majority of ill children; however, a common source of contaminated food or water was not identified. In 2003, an outbreak affected 42 children at a nursery school in Nara, 16 of which were confirmed by culture as serogroup O:8, biovar 1B; the same strain was isolated from a cold salad served to them the week before.[93]

It is noteworthy that sustained person-to-person transmission does not occur with yersiniosis. Rare nosocomial outbreaks (described in Section 5.9) suggest that person-to-person transmission may have occurred, and thus that routine enteric precautions are warranted. The powdered milk outbreak reported above suggests that an infected foodhandler may be a source, again suggesting that routine foodhandler precautions are warranted.

5.3. Geographic Distribution

Y. enterocolitica has been isolated from humans in many countries of the world but is found most frequently in cooler climates. Yersiniosis is most commonly reported in northern European countries, or in New Zealand in the Southern Hemisphere, and is rarely reported in tropical countries.[66] This distribution may reflect differences in frequency of infection in food animal reservoirs and in culinary practices, as well as more intensive surveillance and appropriate culturing techniques in these areas.[94] The infrequent occurrence of *Y. enterocolitica* in some areas of the world may be due in part to culinary habits, such as avoidance of pork in Muslim countries.

Differences in the distribution of the different serotypes of *Y. enterocolitica* isolated from humans appear to be disappearing. Serogroup O:3 biovar 4 is the most widespread in Europe, Japan, Canada, Africa, North America and Latin America. Serogroup O:9 biovar 2 is the second most common in Europe, but its distribution is uneven; while it accounts for a high percentage of the strains isolated in France, Belgium, and the Netherlands, only a few strains have been isolated in Scandinavia.[95] The relative proportion Serotype O:8 was first reported in Europe in the mid-1980s, when a study in the Netherlands showed 25% of all infections were caused by serotype O:8.[37] Until the 1990s the most

frequently reported serogroups in the United States were O:8 followed by O:5,27. In more recent years, serogroup O:3 has been on the increase in the United States in both endemic and epidemic infections.[96] This suggests that the epidemiology of yersiniosis in the United States has evolved into a pattern similar to the picture in Europe, where serogroup O:3 predominates.

5.4. Temporal Distribution

A clustering of cases during the cold fall and winter months has been reported in some European countries, such as Belgium.[73] A similar seasonality has been reported for the frequency with which *Y. enterocolitica* is isolated from swine and from pork, so the seasonality of infections in the swine reservoir may drive the seasonality of infection in humans.[97–99] The association of *Y. enterocolitica* with cooler seasons and temperate climates may be linked to the organism's ability to multiply at low temperatures (see Section 4). In the United States, a study from the early 1990s documented a sharp increase in the number of cases in winter months (December, January, February).[75] More recently, this sharp winter peak in cases has recurred annually (See Figure 1).[67] In Australia, one study found that the peak isolation period for *Y. enterocolitica* from children was in the warm months of December – March.[100]

5.5. Age

Although *Y. enterocolitica* causes a number of clinical syndromes that vary with the age, sex, and health of the host, children appear to be preferentially affected. Of 359 infected patients reported by Mollaret, 47% were between 1 and 5 years of age.[101] Vandepitte and Wauters reported 1,711 cases of *Y. enterocolitica* infection occurring in persons less than 1 month to 85 years old; approximately 20% of all cases occurred within the first year of life.[10] Nearly 80% of patients were less than 10 years old. In the United States, the incidence of infection is greatest in the age group less than 1 year old, among whom the incidence is 25/100,000, compared to 2.7 per 100,000 for children aged 1–4, 0.4 among persons aged 5–59 years, and 0.5 per 100,000 among persons aged 60 or more.[67] In a review of 142 patients with stool cultures yielding *Y. enterocolitica* at a Children's Hospital in the United States, the median age of patients was 5 months with an age range of 18 days to 12 years. Of these children, 85% were 12 months of age or younger.[102] The illness most commonly associated with *Y. enterocolitica* in children less than 5 years old is a febrile diarrheal illness, indistinguishable from that caused by other enteric pathogens (see Section 8.1). Infants less than 3 months old with *Yersinia* enteritis are at greater risk for bacteremia.[102,103] Children over 5 years old are more likely to develop symptoms that mimic those of acute appendicitis. A dramatic difference in age-specific incidence was noted when patients with enteritis were compared with those with the appendicitis-like syndrome.[104] While 80% of all patients with enteritis were less than 5 years old, those with a pseudoappendicitis syndrome were most frequently older children and adults. Most extraintestinal manifestations of *Y. enterocolitica* infection, including the postinfectious sequelae, are more frequently found in adults than in children. In hospital outbreaks, it has been noted that the old and the very young were more often infected and may be predisposed by age and physical condition, including immune status.[105]

5.6. Sex

In general, rates do not differ by sex. In most studies, males have slightly outnumbered females in all age groups of

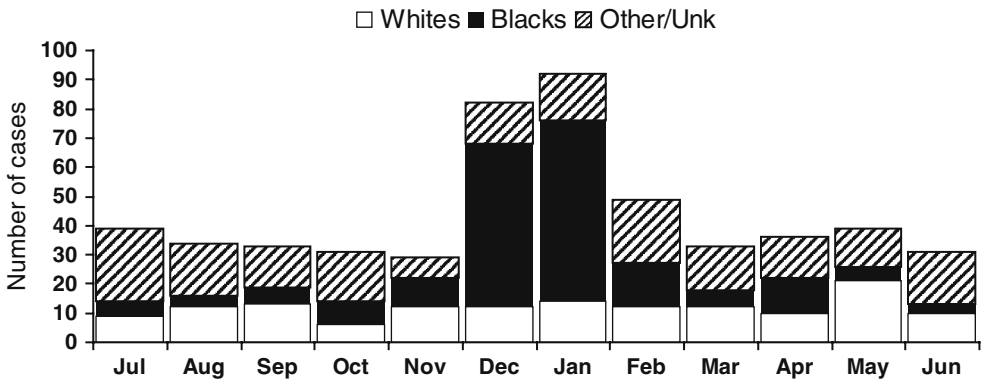


Figure 1. Reported cases of yersiniosis, by month and ethnicity, FoodNet catchment area, United States, 1996–1999, adapted from [67].

patients with *Y. enterocolitica* diarrhea. For example, in one Belgian study, the sex distribution of patients with *Y. enterocolitica* showed more males (54.7%) relative to females.[73] Surveillance data in New Zealand for 2005 showed higher incidence in males (11.8 per 100,000) than females (9.4 per 100.000).[72] In FoodNet, the rates for 2004 were 0.47 per 100,000 in males and 0.39 in females, though these rations are reversed in other years.[19] The sexes tend to be equally affected in outbreaks of mesenteric adenitis. Erythema nodosum is seen more commonly in females.[106]

5.7. Occupation

The organism has been found frequently in samples from swine in slaughterhouses, where it may readily be transferred to employees at all stages during slaughtering and processing (see Section 6). Seroepidemiological studies have indicated that occupational exposure to pigs is a risk factor.[107,108]

5.8. Race and Ethnicity

Race and ethnicity are important because they may reflect differences in specific exposures. In the United States, the incidence of yersiniosis is low among whites (0.5 per million), whereas among African-Americans the incidence of infection is higher (3.4 per 100,000).[67] The highest rates of all were seen African-American infants among whom the incidence of culture-confirmed infections was 143 per 100,000. The winter peak was largely driven by infections in the African-American population, probably because of the seasonal exposure to chitterlings (Figure 1). Rates of infection have been shown to be lower in Muslim populations, which avoid pork.[69] In New Zealand, the highest rates of infection (20.8 per 100, 000) were reported among those of ethnic backgrounds other than European, Maori, and other Pacific Peoples, followed by those of European ethnicity (9.1 per 100,000). The lowest rates were reported among Maori (4.9 per 100,000) and Pacific Peoples.[72]

5.9. Occurrence in Different Settings

Y. enterocolitica among hospital populations typically occurs as sporadic illness and very rarely as outbreaks (Table 2). Some underlying health conditions, such as iron overload and HIV infection, can predispose persons to yersiniosis. Excess iron favors growth and virulence of this iron-dependent organism. The first nosocomial outbreak of *Y. enterocolitica* was reported from Finland in 1973 and involved six hospital employees who became ill approximately 10 days after a schoolgirl with *Y. enterocolitica* enteritis was admitted to their hospital. Spread of the infection, in this and other hospital outbreaks, appeared to be from the index patient to the staff and subsequently from person to person.[109] Another nosocomial outbreak occurred in Canada in 1980, in which nine patients in total were identified, some only by enrichment culture, with possible transmission from the index patient through fomites.[105] In a study of sporadic *Yersinia* infections in hospitalized patients conducted 1987–1990, it was reported that 5 of 18 had developed diarrhea after being hospitalized for unrelated conditions, and thus were likely to represent nosocomial infections.[110]

Outbreaks have been reported among children attending institutions for collective care in eastern Europe, in which a foodborne source was suspected, though not proven.[111] A number of outbreaks have occurred in schools in Japan, without an identified source.[91]

5.10. Socioeconomic Factors

There is little published information suggesting that socioeconomic factors affect the occurrence of *Y. enterocolitica* infection.

6. Mechanisms and Routes of Transmission

Y. enterocolitica can be transmitted through food, water, and occasionally by contact with animals. Rare transfusion-associated cases have also been reported. Transmission by person-to-person contact is also rare. Foodborne transmission is the most common route for sporadic infections and is predominantly associated with consumption of pork. An epidemiological case–control study of sporadic yersiniosis in Belgium documented the importance of consumption of undercooked or raw ground pork, often in the form of raw pork meatballs consumed informally in the kitchen during food preparation.[69] In Norway, an epidemiological case–control study showed that cases of yersiniosis were strongly associated with eating undercooked pork and sausage products and that they generally preferred their meat to be either raw or rare, though very few reported actually eating raw pork.[112] In New Zealand, an epidemiological case–control study of sporadic cases also showed a strong association with eating pork, though the extent of cooking was not established.[113] In 1988–1989, as noted above, an outbreak of infection due to serogroup O:3 among African-American infants in the United States showed that

preparation of raw pork intestines as a holiday food known as chitterlings by the infant's caregiver was significantly associated with illness.[88] Since that outbreak, similar winter clusters have been investigated, with similar findings. Surveys in four cities in the winter of 1989–1990 identified clusters of infections around the holidays of Thanksgiving, Christmas, New Years, and Martin Luther King Day.[96] In 2001, a cluster of 12 cases of *Yersinia* gastroenteritis in black infants was investigated in Tennessee, all of which were exposed to preparation of chitterlings, compared to only 35% of ethnicity and age-matched controls.[114] Exposure was often indirect, and was not necessarily through the caregiver, leading the authors to recommend that chitterlings be routinely irradiated. A cluster of nine infants in Chicago with *Y. enterocolitica* gastroenteritis were investigated in the winter of 2002–2003; of these eight had exposure to chitterlings, and serotype O:3 was isolated both from children and from chitterlings.[115] Raw pork contamination is particularly likely for offal, and ground pork, and strains isolated from pork are difficult to distinguish from those isolated from humans.[3,33]

Untreated drinking water can be another source of sporadic yersiniosis. Consumption of untreated surface water was identified as a risk factor for infection with serogroup O:3 in the case–control study in Norway.[112] Similarly in New Zealand, being connected to the town water supply was significantly protective, suggesting that drinking untreated water was associated with infection.[113]

Y. enterocolitica has been isolated from many domesticated animals and wildlife. Frequently these strains are different from those that have been shown to infect humans. Swine are frequently healthy reservoirs for strains pathogenic to humans, such as serotype O:3 and serotype O:9. In a survey of swineherds conducted in 2000 in the United States, 3.8% of 2,793 fecal samples yielded *Y. enterocolitica* that were ail-positive, and of these 79% were serotype O:3 and had the pYV plasmid.[116] The same survey found that at least one pig was positive on 47% of premises sampled. A survey of pigs at slaughter in Norway found 12% positive for *Y. enterocolitica* serotype O:3, biotype 4, and a similar survey in Sweden found 8% positive.[117] In Sweden, strains of *Y. enterocolitica* isolated from pork had the same pulsed-field gel electrophoresis patterns as those isolated from patients.[118] Dogs, cats, and rodents may occasionally be fecal carriers of serogroups O:3 and O:9, and apparent transmission from dogs and cats to humans has been reported.[119] Rodents were identified as a possible source of infection with serogroup O:8 in Japan.[120]

Some of the most serious, though rare, cases of yersiniosis have resulted from blood transfusions.[121] Ten such cases were reported in the United States between 1991 and 1996 and cases have been reported in other countries as well.[121] A prospective study conducted in the United States between 1998 and 2000 showed that the incidence of transfusion-associated cases of *Yersinia* sepsis was 1 per 23.7 million red cell transfusions.[122] Such cases typically follow transfusion of packed red cells that had been held refrigerated for several weeks after donation. It is presumed that the donors had undetected bacteremia and that the bacteria had the opportunity to multiply during the holding time. Following experimental inoculation into packed red cells at 4°C, *Y. enterocolitica* enter log phase growth in the third week and produce high levels of enterotoxin by 28 days.[123] Just such an asymptomatic bacteremia was detected in a blood donor via routine screening, though the recipient, who was receiving broad spectrum antibiotics at the time of transfusion, had no ill effects.[124]

7. Pathogenesis and Immunity

Human infection with *Y. enterocolitica* is usually acquired by the oral route. The dose required to cause disease in humans is unknown, though one volunteer became ill after consuming 3.5×10^9 organisms.[104] The incubation period typically averages 4–6 days in the outbreak setting. Following ingestion, enteric infection leads to proliferation of *Y. enterocolitica* in the lumen of the bowel and in the lymphoid tissue of the intestine.[44] Secretion of enterotoxin while in the lumen presumably is related to the onset of diarrhea. In the terminal ileum, the bacteria enter the layer of mucous on the gut surface and adhere to the luminal membrane of the enterocytes of the gut wall. Once they penetrate and traverse the enterocyte, they show tropism for the gut-associated lymphoid tissue and multiply in the Peyer's patches and mesenteric lymph follicles, causing a massive inflammatory response. In a self-limited infection, the bacteria are then gradually eliminated. In some persons at higher risk, particularly those with iron-overload syndromes, infection can disseminate from the gastrointestinal tract through the portal vein to produce distant infections throughout the body.

When the localized abdominal pain and fever suggest appendicitis, the patient may wind up on the operating table. The findings at surgery in patients with terminal ileitis are grossly visible, and nearly pathognomonic of yersiniosis. They include extensive mesenteric adenopathy with matted lymph nodes. When tissue is removed and examined microscopically, the pathologist notes cellular infiltrates include lymphoid hyperplasia, epithelioid granulomas, and necrotic suppurative lesions in which the gram-negative organisms are easily demonstrated.

Human infection with pathogenic strains of *Y. enterocolitica* stimulates development of specific antibodies. Although there is considerable evidence that both natural and experimental infection can confer some degree of immunity, the degree to which it is serogroup-specific, and the precise extent and duration of such immunity remain to be determined. In mice, infection with plasmid-containing strains of *Y. enterocolitica* protects against subsequent challenge with *Yersinia pestis*.[125,126]

The postinfectious syndromes appear to be related to the immunologic response to the intestinal infection. The pathogenesis of reactive arthritis caused by *Yersinia* infection is likely to be related to an immune response to *Yersinia* antigens that cross-react with host antigens in susceptible persons. However, the nature of the putative inciting *Yersinia* antigen has not been determined. Host tissue type is the predominant co-factor; HLA-B27 is typically present.[127–129]

T-cells derived from the joint fluid of patients with reactive arthritis have been reported to selectively kill HLA-B27-bearing cells that are also infected with *Y. enterocolitica*.[128] Patients with *Y. enterocolitica*-induced reactive arthritis show a chronically elevated IgA level to a series of virulence-associated, plasmid-encoded proteins (Yops) that are produced by the bacterium.[41,42] Erythema nodosum, the other common post-yersiniosis sequel, does not appear to be associated with HLA-B27.[129]

8. Patterns of Host Response

8.1. Clinical Features

Y. enterocolitica infections in humans can range from asymptomatic carriage to acute gastroenteritis to pseudoappendicitis to a fatal and overwhelming sepsis, and vary with the host as well as with the infecting strain.[2,130] Several organ systems may be involved with *Y. enterocolitica* infection, either by directly or by immunologically mediated mechanisms, and the clinical manifestations can mimic other more common diseases. Patients may present with gastroenteritis, mesenteric adenitis, terminal ileitis, reactive oligoarthritis, erythema nodosum, exudative pharyngitis, or septicemia and coincident complications. Extra-gastrointestinal infections present as osteomyelitis, meningitis, and pulmonary and intra-abdominal abscesses. Other less frequently reported syndromes include carditis, ophthalmitis, glomerulonephritis, hepatitis, pancreatitis, and hemolytic anemia.

Acute, uncomplicated gastroenteritis is by far the most frequently encountered manifestation, particularly in children.[74,103] Acute *Yersinia* gastroenteritis is marked by diarrhea, abdominal pain, fever, and less frequently nausea and vomiting. This syndrome resembles acute infection with other enteric pathogens, and is not readily differentiated clinically, though abdominal pain occurring with *Yersinia* infection is often in the right lower quadrant, which is a diagnostic clue.

Symptoms of *Y. enterocolitica* enteritis typically persist for 1–3 weeks, longer than those of other enteric bacterial pathogens, and occasionally may last for several months.

Tonsillitis is not uncommon in acute yersiniosis, being reported by nearly 20% of patients in sporadic case series and 22% in one outbreak.[69,83131] *Y. enterocolitica* was isolated from throat cultures in 14 patients from this outbreak, illustrating the propensity of the organisms to infect lymphoid tissue such as the tonsils.[132] Since no other cause of acute bacterial diarrhea routinely produces pharyngitis, this symptom can be helpful in suspecting the diagnosis.

The syndromes of mesenteric adenitis, terminal ileitis, or both are more likely in older children and adults[131] (see Section 5.5). In these patients, diarrhea is less prominent, while findings of fever, right lower-quadrant pain, and leukocytosis often mimic those of acute appendicitis. The pseudoappendicitis syndrome has been reported in 3–15% of cases (see Section 5.1). While most cases are probably self-limited, the gut wall can be compromised and severe intestinal disease, small-bowel gangrene, and death have been reported.

A variety of extraintestinal manifestations may accompany enteric infection with *Y. enterocolitica*. Since intestinal symptoms may be mild or asymptomatic, many patients present with syndromes that mimic a host of other non-enteric diseases, and thus present a diagnostic challenge to the physician. The most common extraintestinal form of *Y. enterocolitica* infection is reactive arthritis. A non-suppurative arthritis has been reported most frequently in persons of northern European descent. In Scandinavia, 10–30% of adults with *Y. enterocolitica* infection develop inflammation of the knees, wrists, or ankles a few days to 1 month after the onset of diarrhea.[133] Cultures of synovial fluid cultures are generally negative. Symptoms usually resolve after 1–6 months but may become chronic in some patients.[134] The HLA B27 tissue type is a clearly demonstrated co-factor.[127] Reactive arthritis, sometimes accompanied by conjunctivitis and urethritis, as well as chronic progressive ankylosing spondylitis have been described in individuals with the HLA B27 antigen following *Y. enterocolitica* infection.[129,135]

Erythema nodosum occurs in up to 30% of Scandinavian cases, most of whom are women.[106] In the majority of cases, erythema nodosum resolves spontaneously within a month. This complication has not been linked to HLA type B27. The

causative agents of reactive arthritis and erythema nodosum belong to serogroups O:3 or O:9. These manifestations have been relatively rare in the United States, an observation that has been related to the geographic distribution of serogroups. However, an increase in cases with postinfectious manifestations of yersiniosis would not be unexpected, parallel to the emergence of serogroup O:3 in the United States (see Section 5.3).

Septicemia due to *Y. enterocolitica* is seen almost exclusively in infants or in individuals with underlying disease.[2] Those with cirrhosis and disorders of iron excess are particularly predisposed to infection and increased mortality. The cases of septicemia and shock that follow transfusion with packed red cells illustrate the severe nature of the infections in compromised recipients.[121] The blood donors for these cases are typically asymptomatic, which means that asymptomatic bacteremia among health adults occasionally occurs.

Transient carriage and excretion of both pathogenic and non-pathogenic *Y. enterocolitica* may occur following exposure to the bacterium. High rates of asymptomatic carriage have been reported in connection with outbreaks.[89] However, inapparent infection with *Y. enterocolitica* was detected in less than 1% of individuals in large surveys.[78] In patients with *Y. enterocolitica* enteritis, the organism may be excreted in low numbers in the stools for a long time after symptoms have resolved. In a study of Norwegian patients, convalescent carriage of *Y. enterocolitica* O:3 was detected in 47% of 57 patients.[131] In these persons, the organism was carried for a median of 40 days (range, 17–116 days).

Intestinal infection with *Y. enterocolitica* is usually self-limited and antimicrobial treatment is not routinely needed. Life-threatening sepsis, however, requires treatment. Antibiotic treatment has not been shown to shorten the duration of acute gastroenteritis due to *Y. enterocolitica*. In a retrospective case series from Norway, treatment was not associated with a decreased duration of illness (18 days versus 21 days).[131] No clinical benefit was demonstrated in a small prospective, placebo-controlled trial of trimethoprim–sulfamethoxazole in Canadian children.[136] However, the organism rapidly disappeared from the stools in both studies. There is also no evidence that early antimicrobial therapy reduces the frequency or severity of chronic sequelae.[137] Treatment of life-threatening infections with fluoroquinolones or third-generation cephalosporins has been associated with a decrease in mortality.[138]

Y. enterocolitica have been shown to be susceptible in vitro to aminoglycosides, chloramphenicol, tetracycline, trimethoprim–sulfamethoxazole, and third-generation cephalosporin antibiotics.[2,139,140] Most strains produce a β-lactamase, making them resistant to penicillin, ampicillin, and first-generation cephalosporins.[141] The recent emergence of reduced susceptibility to fluoroquinolones in *Y. enterocolitica* in southern Europe may herald coming challenges to treatment of life-threatening infections.[142]

8.2. Diagnosis

Diagnosis depends on isolating the organism from a clinical specimen, such as stool or blood. *Y. enterocolitica* can be easily isolated from sterile sites with routine bacterial culture methods The diagnosis of *Y. enterocolitica* gastroenteritis requires a degree of clinical suspicion and specific consideration in the clinical laboratory, so that selective isolation media are used for stool culture (see Section 3.4.1). Enterocolitis caused by *Y. enterocolitica* cannot easily be differentiated clinically from acute infections due to other enteric bacterial and viral pathogens.[2,131] However, *Y. enterocolitica* infection can present with distinguishing features that suggest the diagnosis. Tonsillitis and localized right lower-quadrant abdominal pain are diagnostic clues. A recent history of consumption of undercooked pork, drinking untreated water, or contact with pigs may suggest the diagnosis of yersiniosis (see Section 6). The diagnosis should also be considered when joint pains or erythema nodosum appears in persons with previous diarrheal illness, recurrent diarrhea, or abdominal pain.

Serological diagnosis can be useful in research studies to determine the role of *Y. enterocolitica* in postinfectious syndromes and other late manifestations (see Section 3.4.2). Several limitations of serodiagnosis make it problematic for use in clinical practice, including cross-reactions with other bacterial species and the prevalence of seropositive individuals in the healthy population.

9. Control and Prevention

9.1. General Concepts

Basic tenets of safe food production and preparation are critical to preventing these infections. Preventive measures that reduce contamination and improve hygiene during all stages of pig and pork processing are essential to reduce infection with serogroups O:3 and O:9. The organism may be difficult to control at the farm level. At slaughter, routine visual inspection will not detect the organism, as swine are healthy carriers of *Y. enterocolitica*, and bacteria from the oral cavity or intestinal contents may easily contaminate the carcasses and the environment in the slaughterhouse. Improved hygiene at critical control points is needed, with

special attention during: (1) circumanal incision and removal of the intestines; (2) excision of the tongue, pharynx, and particularly the tonsils which often harbor *Yersinia*; (3) meat inspection procedures that involve cutting into the mandibular lymph nodes; and (4) deboning of head meat.[34,45,117] Changes in slaughtering procedures, including technological improvements, may be required to reduce contamination during these activities. Routine treatment of raw pork and raw pork products with electron beam irradiation would greatly decrease infections arising from this route.[143]

Routine pasteurization of milk is another fundamental prevention measure for yersiniosis and many other infections. Milk-associated outbreaks of yersiniosis illustrate the need for careful sanitation measures to prevent contamination of milk after pasteurization, through contamination of the dairy environment and of empty containers before they are filled with milk. Particular care is needed in dairies that feed pigs leftover "swill" milk, as the transit of milk cans to and from the pigs can connect milk production and pig pens.[83,87]

Preventive and control measures should also focus on informing all people involved in production, procession, and final preparation of food about the importance of good hygienic practices. Strict hygiene is particularly necessary because *Y. enterocolitica* is able to propagate at temperatures approaching 0°C (Section 4).

The consumer can prevent infections through wise food choices and good kitchen hygiene. Infections can be prevented by not consuming raw unpasteurized milk, and raw or undercooked pork. Separating the two tasks of caring for an infant and simultaneously preparing raw pork intestines or other raw pork dishes can prevent illness in the infant.[88,114]

Y. enterocolitica is sensitive to chlorination, so proper treatment of drinking water should eliminate the risk of infection from this source.

Testing units of packed red cells for the presence of bacterial endotoxin, and limiting the shelf-life of packed red cells may be helpful in preventing transfusion-associated sepsis.[122]

9.2. Antibiotic and Chemotherapeutic Approaches to Prophylaxis

Antibiotic or chemotherapeutic approaches to prophylaxis are not indicated.

9.3. Immunization.

Vaccines against *Y. enterocolitica* infections have not been developed. The high frequency of immune-mediated complications of yersiniosis in humans has tempered enthusiasm for such research. Attenuated strains have been used as a means of delivering other antigens in experimental vaccine models.[144,145]

10. Unresolved Problems

Despite better understanding of the biological properties of the organism and its unique geographic and environmental distribution, many questions about the ecology, epidemiology, and pathogenicity of *Y. enterocolitica* remain unanswered. In many parts of the world, its importance is simply unknown. In New Zealand, a high incidence remains unexplained. To date, the important declines in incidence in Belgium and the United States suggest that control is certainly possible, though in the latter case the actual reasons for the decline are obscure. The development of standardized and approved laboratory methods is needed to allow optimal recovery of all pathogenic serogroups and to provide adequate differentiation between pathogenic and non-pathogenic variants. Standardized practical molecular subtyping methods that allow better differentiation within species and serogroup are needed to assist in public health surveillance, outbreak detection, and epidemiological investigation.

Although considerable progress has been made in our understanding of the routes of transmission and reservoirs for *Y. enterocolitica*, well-designed epidemiological investigations, particularly case-control studies, are needed to identify risk factors and determine their relative importance in endemic areas. Intervention studies are required to assess the efficacy of specific and nonspecific control and preventive measures to reduce colonization on the farm, contamination at the slaughterhouse, growth during storage, and spread in the kitchen. The strong association of the pathogenic strains with the swine reservoir suggests that specific interventions to immunize swine or otherwise reduce transmission among pigs may offer benefits to human health.

Great strides have been made in our understanding at the molecular level of the mechanisms by which *Y. enterocolitica* causes disease. However, further clarification of the role of the virulence determinants is needed. The evolutionary links between *Y. enterocolitica*, *Y. pestis*, and *Y. pseudotuberculosis* suggest that this genus may offer more general answers to the questions about how pathogens evolve, how they develop specific tissue tropisms, and how they become able to evade host immune response. All three share mammalian hosts, an ability to infect via the gastrointestinal tract, and cross-protective antigens. The historical puzzle of the apparent resistance of some medieval European populations to bubonic plague might be explained by cross-protection due to infections with other endemic *Yersinia*. The persisting clinical puzzles of human yersiniosis need to be unraveled,

including the role of iron in enhancing disease; the relationship of *Y. enterocolitica* to the immune system to produce a diversity of autoimmune phenomena; the potential benefit of early diagnosis and treatment; and the nature, efficacy, and duration of natural immunity.

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